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Neural Circuits

Principal Investigator: ABELIOVICH, ASA
Grant Number: 5R01NS046659-02
Title: Molecular and Cellular Analyses of Parkin Function

Abstract: Defective protein degradation through the ubiquitin proteasome pathway (UPP) has been hypothesized to play a central role in neurodegenerative disorders such as Parkinson's disease (PD). Mutations in Parkin, a putative ubiquitin ligase component, cause a familial, autosomal recessive form of PD characterized by midbrain dopamine neuron loss. It has therefore been hypothesized that inefficient degradation and consequent toxic accumulation of Parkin ubiquitination substrates underlie the loss of dopamine neurons in autosomal recessive Parkinson's disease. We further hypothesize that Parkin may play a direct role in regulating neuronal survival in the CNS. We propose to use molecular and cellular tools to investigate the mechanism of Parkin action in protein ubiquitination and neuronal survival. Our preliminary data indicate that Parkin associates in a multiprotein ubiquitin ligase complex with 2 previously characterized ubiquitin ligase components, the F-box/WD repeat-containing protein hSel- 10, and Cullin-1 (Cul 1). Furthermore, hSel-10 serves to direct this complex to specific substrates including Cyclin E, a putative regulator of neuronal apoptosis. We will test the hypotheses that (1) auxiliary components of the Parkin ubiquitin ligase complex serve to regulate or target this activity, and (2) that, in Parkin-associated familial PD, premature neuronal death is a consequence of defective ubiquitination and the accumulation of neuronal apoptosis-related Parkin complex substrates. -

Principal Investigator: ALBUQUERQUE, EDSON
Grant Number: 5R01NS041671-04
Title: Nicotinic Receptors in Septally Innervated Hippocampus

Abstract: The dysfunction and degeneration of the nicotinic cholinergic system in the brain are integral physiopathological indicators of one of the most socially impacting neurological disorders, Alzheimer's disease (AD). In AD, the permanent loss of cholinergic neurons and nicotinic receptors (nAChRs) in brain areas that process cognitive functions, particularly the hippocampus and the frontal cortex, correlates well with the decline in cognition and memory. To date, treatment of patients with AD relies heavily on the use of acetylcholinesterases. These drugs, by increasing function of the cholinergic system, partially reverse the symptoms of AD patients. Recently, clinical trials have shown that nicotinic agonists (including nicotine) and drugs that allosterically potentiate the activity of nAChRs are more effective for treatment of patients with AD. The mechanisms underlying the effectiveness of these drugs remain unknown, because there is very little information on how function and expression of neuronal nAChRs in the brain are regulated by cholinergic afferents. In addition, detailed analysis of regulation of nAChR expression and function in the hippocampus by septal cholinergic afferents has been limited by the lack of a viable biological preparation that closely resembles the nicotinic cholinoceptive hippocampal system in vivo. Our initial characterization of the nicotinic properties of hippocampal neurons in organotypic, hippocampal and septal-hippocampal cultures constitutes the mainstay of the present proposal, as it establishes the septal-hippocampal co-cultures as an excellent model for in vitro study of the influences of septal innervation on nAChR expression in the hippocampus. Thus, this proposal is designed to use convergent, multidisciplinary approaches to address the central hypothesis that septal innervation and nicotine dynamically modify the hippocampal cholinergic system. The first goal of this study is to use electrophysiology, confocal microscopy, ligand binding and immunocytochemistry to determine whether septal innervation alters the nicotinic properties of different types of hippocampal neurons during development in organotypic cultures. The second goal is to use electrophysiological assays, recombinant DNA technology and "knock-out" mice, which have a null mutation in the gene encoding alpha7 nicotinic receptors, to study nAChR targeting and to investigate the motifs in the nAChR subunits that account for final receptor targeting in hippocampal neurons. The final goal is to use electrophysiological, biochemical and molecular biological techniques to evaluate how nicotine affects alpha7 and alpha4beta2 nAChR expression in the hippocampus. The results of these studies will have far

Principal Investigator: ALLOWAY, KEVIN D
Grant Number: 5R01NS037532-06
Title: Corticostriatal Influences on Neostriatal Processing

Abstract: The neostriatum and related parts of the basal ganglia contain functional channels that separately process prefrontal, limbic, oculomotor, and sensorimotor information received from the cerebral cortex. Although the specific cortical areas that activate each functional channel are different, each channel contains the same basic input-output pattern of connections. By using cutaneous stimuli to activate corticostriatal projections from somatosensory cortex, we can characterize the pattern recognition properties of neostriatal neurons and determine how neural activity in the sensorimotor channel is coordinated during sensory stimulation. Hence, this paradigm presents a unique opportunity for understanding the rules that govern the dynamic operation of corticostriatal circuits across all functional channels because there are no accepted methods for directly activating limbic or prefrontal channels in a controlled, naturalistic manner. The anatomic and physiologic properties of the neostriatum represent a significant issue in contemporary neuroscience because this brain region has been implicated in Parkinson's disease, Huntington's chorea, Tourette's syndrome, and schizophrenia. In this project we will use anterograde tracing methods to test the hypothesis that corresponding representations in the primary and secondary somatosensory cortical areas send convergent projections to the neostriatum. We will also use retrograde tracing techniques to verify previous results indicating that corticostriatal projections from somatosensory cortex have an anisostropic organization. We will also compare corticostriatal and corticopontine projections to determine if the corticopontine projections follow the same principles of organization that have been identified in the corticostriatal system (ie. Principles of Cortical Proximity, Somatotopic Homology, and Behavioral Cooperativity). Finally, we will record neuronal activity in multiple parts of the cerebral cortex and neostriatum so that we may analyze neuronal interactions between the cortex and neostriatum. These physiology studies will determine whether a primary function of neostriatal neurons is to detect synchronized activity among functionally-related cortical areas. -

Principal Investigator: ANDERSON, MARJORIE
Grant Number: 5R01NS044565-03
Title: Deep Brain Stimulation in Parkinson's Models

Abstract: Although high-frequency deep brain stimulation (HF-DBS) in the globus pallidus or subthalamic nucleus has become a common technique used to treat drug-resistant symptoms of Parkinson's disease, the mechanisms by which HF-DBS exerts its effects are unknown. In the proposed studies, the ability of chronic administration of the insecticide rotenone, to produce an animal model of Parkinson's disease will first be tested in monkeys. Using PET imaging now available in the University of Washington Regional Primate Research Center, changes in dopamine innervation after administration of rotenone will be measured using a marker of the monoamine vescicular transporter that is present in dopaminergic nerve terminals. These changes will then be correlated, over time, with changes in behavior and with electrophysiological changes in the rate and pattern of discharge of neurons in basal ganglia-receiving areas of the thalamus. This model will then be used to couple the electrophysiological effects of HF-DBS, which can be recorded from basal ganglia-receiving neurons of the thalamus, to the stimulation-induced changes in regional metabolism in the cortex and thalamus. PET imaging with the metabolic marker, [8-F] flurodeoxyglucose (FDG), will be used to measure metabolism. This technique has generally shown a relative hypermetabolism in the globus pallidus and thalamus of humans with Parkinson's disease and a relative hypometabolism in areas of the frontal cortex. Changes reported to be induced by HF-DBS have been mixed however. The combination of electrophysiology and metabolic imaging will allow us to address some of the discrepancies from the human literature. Special attention will be paid to the development of abnormal patterns of bursting behavior in the thalamus of monkeys treated with rotenone, as well as the effect of HF-DBS on burst behavior. This will test the hypothesis that some of the symptomatology of Parkinson's disease, and its relief using HF-DBS, is a consequence of abnormal patterns of activity in basal ganglia-thalamic-cortical circuits.-

Principal Investigator: ASSAD, JOHN A
Grant Number: 5R01NS041000-05
Title: BASAL GANGLIA FUNCTION--BASIC MECHANISMS AND EFFECTS

Abstract: The basal ganglia (BG) are a set of subcortical nuclei that play a crucial role in the control of voluntary movements. Their importance is underscored by diseases of the BG, such as Parkinson's disease, which compromise the initiation and execution of voluntary movements. While much is known about the general organization of the BG, fundamental questions remain about their role in the normal control of movement. These questions are particularly relevant given the renewed interest in restorative neurosurgical procedures, such as chronic electrical stimulation. that target the BG to relieve Parkinsonian symptoms. The main goal of this is to understand the role of the BG in the normal control of movement, using the awake behaving macaque monkey as an experimental system. The first aim addresses an intriguing paradox about the BG: while diseases affecting the BG cause problems with initiating voluntary movements, most neurophysiological studies have found that neuronal activity in the BG occurs too late to play a role in movement initiation. However, in most of these studies the movements were in response to an external sensory stimulus. There is evidence from Parkinsonian patients that stimulus-cued movements are less severely affected than self-initiated movements. We will thus examine whether the BG play a special role in self-initiated movements - self-initiated with respect to either when a movement is made or which movement is made. The second aim addresses the roles of the direct and indirect BG pathways. The output of the BG is influenced by two distinct pathways with opposing effects on movement: a direct pathway from the striatum which facilitates movement, and an indirect pathway via the subthalamic nucleus (STN) which inhibits movement. While the identification of these pathways has provided a useful framework for understanding movement disorders, many questions remain about their roles in normal movement. We will test one hypothesis, that the two pathways may act in concert to "select" a specific movement among competing possibilities of movement, by examining how neurons in the output nuclei of the BG are affected by electrical inactivation of the STN. For this purpose, it will be necessary to examine the neuronal effects of electrical stimulation in the STN. Little is known about the neuronal effects, even though STN stimulation is now being used to treat Parkinsonian symptoms in human patients. We will directly measure the neuronal effects of electrical stimulation in the STN, and examine how these effects vary with the parameters of stimulation. For this we will develop and test new multielectrode techniques for recording from and

Principal Investigator: BAUDRY, MICHEL
Grant Number: 1R01NS048521-01A1
Title: Calpain inhibitors in models of Parkinson's disease

Abstract: Parkinson' s disease is a neurodegenerative disease that specifically affects dopaminergic neurons in the substantia nigra. Although several hypotheses have been proposed to account for the specificity of the neurodegenerative features of the disease, the exact cause of the disease remains to be elucidated. Significant advances in our understanding of the possible causes of the disease were provided by the serendipitous discovery that a neurotoxin, 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP), elicits a pattern of neurodegenerative features in humans and experimental animals identical to that seen in patients with Parkinson' s disease. A potential target to prevent neurodegeneration in Parkinson' s disease is the calcium-dependent protease calpain. Calpain levels are elevated in post-mortem substantia nigra of patients with Parkinson' s disease, MPP+ neurotoxicity in granule cell cultures is associated with calpain activation and blocked by calpain inhibitors, and calpain has been implicated in several neurodegenerative diseases. We have recently obtained a series of novel and potent calpain inhibitors and have demonstrated their potency in preventing NMDA-induced calpain activation in cultured hippocampal slices. The current proposal is aimed at testing the hypothesis that calpain activation plays a critical role in animal models of PD and that calpain inhibitors are neuroprotective in these models. We will first determine the potency and efficacy of calpain inhibitors to prevent MPTP toxicity in cultured slices from rat mesencephalon. We will then use structure activity relationship in conjunction with additional assays to identify the best inhibitors to be tested in in vivo models. Finally, we will test the hypothesis that calpain is activated and that calpain inhibitors are neuroprotective against MPTP-mediated neurotoxicity and behavioral impairments in vivo in C57BI/6 mice, and against rotenone-mediated neurotoxicity in rats. Conversion of the pro-apoptotic factor Bid to its active, truncated form tBid will be tested as part of the mechanisms by which calpain activation induces cell death. These studies will test the hypothesis that calpain inhibitors might prevent neurodegeneration not only in Parkinson' s disease but also in a variety of conditions resulting from exposure to environmental toxins. Finally, because calpain has also been implicated in the mechanisms underlying Amyotrophic Lateral Sclerosis (ALS), our proposal could lead to significant advances in the treatment of this neurodegenerative disease as well. -

Principal Investigator: BEVAN, MARK D
Grant Number: 5R01NS041280-05
Title: DYNAMICS OF GABAERGIC INHIBITION IN THE SUBTHALAMUS

Abstract: The excitatory subthalamic nucleus is a major driving force of neuronal activity in the basal ganglia of animals and humans under resting conditions, during voluntary movement and in idiopathic and experimental models of Parkinson's disease. The activity of the subthalamic nucleus is regulated by GABAergic inhibition from the reciprocally connected external globus pallidus. The overall objective of this research is to determine the principles that underlie GABAergic inhibition in the subthalamic nucleus in health and in Parkinson's disease. Perforated patch, whole-cell or extracellular recordings of subthalamic neurons in slices will be used to examine the effects of GABAergic inhibitory postsynaptic potentials/currents (GABA ipsps/ipscs) on spontaneous or driven rhythmic firing. Bursts of GABA ipsps/ipscs will be evoked in subthalamic neurons to determine whether GABA acting at -A and/or -B receptors can produce rebound burst firing in subthalamic neurons. The role of dopamine in the regulation of GABAergic synaptic transmission in the subthalamic nucleus will be examined. Slices containing an intact subthalamic nucleus-external globus pallidus network in the presence of dopamine agonists or antagonists will be studied to determine whether spontaneous or evoked burst firing in subthalamic neurons can trigger low frequency oscillatory activity. Single pallidal neurons will be filled in vivo using juxtacellular labelling and their synaptic boutons in the subthalamic nucleus will be plotted in three dimensions and examined by light and electron microscopy to determine whether neighbouring neurons receive common inputs. The degree of divergence and convergence in the pallidal projection will be determined from stereological estimates of GABAergic terminals in the subthalamic nucleus and the known numbers of neurons in the network. -

Principal Investigator: BREDESEN, DALE E
Grant Number: 5R01NS033376-07
Title: Novel apoptotic pathway activated by misfolded proteins

Abstract: Neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS) and prion protein diseases all feature misfolded proteins and their aggregates, which appear to play a role in disease pathogenesis. However, the mechanism(s) and pathways by which misfolded proteins couple to the cell death program is poorly understood. We have recently found that misfolded proteins, which trigger endoplasmic reticulum stress (ER stress), induce a novel intrinsic apoptotic pathway that is independent of Apaf-1 and mitochondria (Rao et al., 2001; Rao et al., 2002a; Rao et al., 2002b). In order to define the molecular requirements of this pathway, we have developed a cell-free system of ER stress-induced apoptosis. In this system, microsomes isolated from cells lacking ER stress fail to activate cytosolic extracts, whereas microsomes isolated from cells undergoing ER stress activate caspases-3 and -9 in cytosolic extracts. Using a set of complementary approaches including protein purification procedures, 2D MALDI-TOF/nano-ESMS (two-dimensional matrix-assisted laser desorption ionization-time of flight/nanoelectrospray mass spectrometry), immunodepletion of candidate proteins, and an RNA interference (RNAi) approach, we have identified the initial candidate biochemical mediators of this novel apoptotic pathway, and here we propose to continue to identify the components of this ER stress-induced apoptotic pathway. Because an understanding of the relationship between the accumulation of misfolded proteins, cellular stress response, and cell death programs should facilitate the development of new therapeutic strategies for neurodegenerative disorders that feature misfolded proteins, we propose to integrate the findings from our cell work with an animal model of ALS, and address the following questions: (1) What are the proteins that mediate ER stress-induced cell death? (2) Within the set of relevant proteins that are differentially expressed, what are the crucial proteins for cell death induction? (3) Is there evidence of activation of the ER stress-induced apoptotic pathway in transgenic mice expressing mutant (vs. wild type) CuZnSOD? (4) Does recombinant mutant CuZnSOD protein induce normal organelles to initiate a specific cell death pathway? We believe that the results of the proposed experiments may offer insight into the pathogenesis of neurodegenerative disorders that feature misfolded proteins, and should enhance the usefulness of our system for development of therapeutics.-

Principal Investigator: BURKE, ROBERT E
Grant Number: 2P50NS038370-06
Title: Mechanisms of dopamine neuron degeneration

Abstract: Parkinson's disease (PD) is a prevalent and disabling neurological disease characterized by the progressive loss of motor control due to the degeneration of dopamine (DA) neurons of the substantia nigra. Among neurodegenerative diseases, PD has served as a model for the development of novel therapeutic approaches: administration of neurotransmitter precursors (levodopa), cell implantation, and more recently, deep brain stimulation. As important and effective as these advances have been, they only relieve symptoms; none stop the progression of the disease. In order to develop therapies which halt the progression of the disease, we need to achieve a better understanding of the pathogenesis of DA neuron degeneration. This submission represents a competing continuation application for a Morris K. Udall Parkinson's Disease Research Center of Excellence awarded to Columbia University in 1999. This renewal consists of four projects devoted to a single integrating theme: to understand the molecular and cellular mechanisms of dopamine neuron degeneration. While there are many worthy hypotheses of pathogenesis, the subprojects of this proposal will focus on four major current themes in the pathogenesis of PD, related to the roles of: (1) Abnormal intracellular protein degradation; (2) Inflammatory pathways; (3) Programmed cell death (PCD); and (4) Oxidative injury. In Project 1, Dr Serge Przedborski will evaluate the role of cyclooxygenase 2 (COX2) and cytosolic phospholipase A2 (cPLA2) (Theme 2) in mediating dopamine neuron damage in the MPTP model of PD and in human brain samples. In Project 2, Dr David Sulzer will examine in astrocyte and neuron primary cultures the role of chaperone mediated autophagy in the degradation of proteins implicated in PD (Theme 1) and the effect of these proteins on catecholamine sequestration (Theme 4). In Project 3, Dr Robert Burke will use genetic techniques in animal models to examine the roles of the mixed lineage kinases, Akt and JNK in mediating PCD in dopamine neurons (Theme 3), and he will evaluate the functional role of ER stress in initiating cell death (Theme 1). In Project 4, Dr Lloyd Greene will continue to evaluate the functional role of genes identified in the current funding period by SAGE analysis as upregulated following neurotoxin exposure. He will continue his studies of the role of ER stress-related genes (Theme 1) and genes implicated in PCD (Theme 3) in PC12 cells and primary sympathetic neurons, and in living animal models (the latter in collaboration with Drs Burke and Przedborski). He will also examine these transcripts and their protein products in PD brain. -

Principal Investigator: CALLAWAY, JOSEPH C
Grant Number: 5R01NS042276-03
Title: Dendritic Role in Dopamine Neuron Firing

Abstract: Destruction or dysfunction of the dopaminergic neurons of the mesencephalon is believed to underlie a variety of disorders of movement, motivation and mentation, including Parkinson's disease, and schizophrenia. In those disorders, not accompanied by death of the dopaminergic neurons, it is likely that a disruption of the activity patterns of those neurons is an important component of the pathology. Dopaminergic neurons fire in stereotyped modes, controlled largely by calcium currents and by calcium-dependent potassium currents. We will employ calcium-imaging of single neurons injected intracellularly with calcium indicator during whole cell recording in slices visualized by infra-red DIC microscopy. This will allow simultaneous detection of membrane potential at the cell bodies of the dopaminergic neurons and detection of calcium entry in the cell body and dendritic tree. Current models of firing pattern generation by dopaminergic neurons differ in their predictions of the location of calcium entry, and our experiments allow a critical test of these models. Synaptic excitation and local dendritic excitation by applied glutamate or glutamate agonists will be used to test for the local control of dendritic calcium currents by subthreshold excitatory currents. We will measure how action potentials propagating into dopamine cell dendrites contribute to slow oscillations in dendritic calcium levels and resulting calcium dependent potassium current that ultimately control the output firing pattern. Experiments will examine how the extent of dendritic spike propagation regulates pacemaker firing rate and whether modulation of dendritic spike propagation contributes to irregular and burst firing. Calcium channel blockers will be used in conjunction with calcium imaging to establish the types and distribution of calcium channels that contribute to voltage dependent calcium imaging to establish the types and distribution of calcium channels that contribute to voltage-dependent calcium entry in these cells. Finally, immunocytochemistry using antibodies against clones of channel subtypes will also be used to examine the distribution of calcium and calcium dependent potassium channels in the dendritic arbors of dopamine cells and results from will be compared to those from calcium imaging. -

Principal Investigator: CANAVIER, CARMEN C
Grant Number: 5R01NS037963-06
Title: Firing Pattern in Midbrain Dopamine Neurons

Abstract: :(provided by applicant) This work seeks to understand the how the synaptic afferent inputs to midbrain dopamine neurons interact with their intrinsic properties to produce the range of firing patterns exhibited in vivo, and how these firing patterns exert their effects on the target neurons in the striatum. We will first produce a computer model of the dopamine neurons in vitro that replicates the effects of pharmacological manipulations on the regular spontaneous firing that characterizes dopamine neurons in the absence of afferent input, and provides insight into the mechanisms that convert this regular firing into burst firing or irregular firing. Then we will extend the model to the situation in vivo. The model will be used not only to elucidate the key currents, parameters, and mechanisms responsible for the generation and modulation of their electrical activity, but also to suggest therapeutic approaches for Parkinson's disease and other pathological conditions in which dopamine release plays a role. Currently such therapeutic strategies, including maximizing release from surviving or transplanted dopamine neurons, are limited by the inability to replace dopamine in the correct spatial and temporal pattern. Several lines of evidence indicate that not only the firing rate but also the firing pattern of these neurons is significant. Computational models supplemented by the techniques of nonlinear forecasting and nullcline analysis, will used to test our hypotheses about how various pharmacological agents exert their effects on the firing pattern of dopamine neurons, and how these changes in firing pattern might impact their targets in the striatum. We will identify model mechanisms and parameters responsible for characteristics of apamin and NMDA-induced burst firing such as variations in spike amplitude and interspike interval (ISI) as well as depolarization block, identify mechanisms responsible for irregular firing both in the model and in real neurons in vivo and in vitro, formulate a model of burst firing induced by synaptic excitation in vivo, and test our hypotheses regarding the functionality of irregular firing and the role of Dl receptor activation in focusing striatal activity. -

Principal Investigator: CHANG, JING-YU
Grant Number: 5R01NS043441-03
Title: Rat Model of Brain Stimulation in Parkinsonian Condition

Abstract: Deep brain stimulation (DBS) has been used in the clinic to treat Parkinson's disease (PD) during the past decade. The neuronal mechanisms underlying the therapeutic effects of DBS, however, are yet to be clarified. DBS methods have been developed based on the experiments performed exclusively on primate model. Many critical issues regarding the therapeutic effects of DBS need to be addressed using a rodent model. This proposal is aimed at three objectives: first is to establish a rodent model of DBS for Parkinsonian conditions. The rat will be subjected to unilateral 6-hydroxydopamine injection to destroy nigrostriatal dopamine system and thus develop a Parkinsonian motor deficit revealed by treadmill locomotion task. Treadmill will be turned on and off 20 seconds alternatively. Array of ten stimulation electrodes will be implanted in the subthalamic nucleus (STN) and substantia nigra pars reticulata (SNr). High frequency stimulation (HFS) will be applied during the treadmill walking phase. The improvement on locomotion by HFS will be measured and the effects will be compared between STN and SNr stimulations using different stimulation parameters. Second objective is to understand the dynamic neural activity responses in the basal ganglia system during the development of motor deficit by monitoring and comparing the activities from same neurons cross 10 day dopamine depletion period. Chronic multi- channel, single-unit recording technique will be used in this experiment. Sixty-four electrodes will be implanted in the striatum, globus pallidus, STN, and SNr. Extracellular spike activity will be recorded simultaneously in the behavioral rat. This study will test the hypothesis that direct and indirect pathways of basal ganglia will respond in different yet correlated manners during dopamine depletion. Third objective is to study the neuronal mechanisms mediating therapeutic effects of DBS in the behavioral model described above. In addition to the 64 electrodes implanted in the basal ganglia regions mentioned above, eight more stimulation electrodes will be added to target the STN and SNr. The neuronal responses in all four basal ganglia regions during behavioral effective HFS will be recorded and analyzed to reveal the effects of HFS on motor behavioral and associated changes in basal ganglia neuronal activity. This study is designed to address the fundamental mechanisms regarding the effects of DBS on treating PD and the information obtained from this experiment will have direct impact on improving the effects of DBS on PD and other movement disorders.-

Principal Investigator: CHANG, JING-YU
Grant Number: 5R01NS045826-03
Title: Basal Ganglia Neurophysiology during DBS in Rats

Abstract: Parkinson's disease (PD) is a degenerative neurological disorder affecting millions of patients all around the world. Renewed use of the deep brain stimulation (DBS) method provides a new opportunity for treating PD. A key issue to improve the treatment is to fully understand the neural mechanisms underlying the therapeutic effects of DBS. In this proposed study, two unique techniques developed in our laboratory: the chronic multiple-channel single unit recording and rat model of DBS, will be employed to study the neural responses in multiple basal ganglia regions during behaviorally effective DBS in rat model of Parkinsonism. A first objective is to establish a rodent model of DBS in Parkinsonian conditions. The effects of DBS will be evaluated in dopamine lesioned rats performing treadmill locomotion and limb use asymmetry tests. Locomotor d deficits during treadmill walking and imbalance usage of forelimb in vertical exploratory behaviors will develop after unilateral dopamine lesion. High frequency stimulation (HFS) of the subthalamic nucleus (STN) and the substantia nigra pars reticulata (SNr) will then be applied to alleviate these motor abnormalities. The degree of dopamine depletion in the basal ganglia will be detected by immunohistochemical staining of dopamine marker and this result will be correlated with the severity of motor deficits and DBS effects. Second, the basal ganglia neural responses following a dopamine lesion and during behaviorally effective HFS will be examined. Single neural activity and local field potential in the striatum globus pallidus, STN and SNr will be recorded simultaneously in a 64 channel recording system in the rat performing these behavioral tests. Neural responses following dopamine lesion will help us to understand the pathophysiologic process of developing Parkinsonian syndromes while the neural responses during behaviorally effective HFS will shed light on how DBS can restore normal information processing in the basal ganglia neural circuits that are disrupted following dopamine lesion. Several important improvements on recording and stimulation techniques will be made in cooperation with Biographic Inc. to achieve optimal conditions for high frequency stimulation and artifact free recording. The goal of this study is to explore the basic neural mechanism underlying the therapeutic effects of DBS and the knowledge obtained form this study will help us to improve the clinical treatment of PD with DBS method. -

Principal Investigator: CHAO, MOSES V
Grant Number: 2R01NS021072-19
Title: Molecular Analysis of Nerve Growth Factor Action

Abstract: Neurotrophins represent an important family of polypeptide growth factors which influence the proliferation, differentiation, survival and death of neuronal and non-neuronal cells during vertebrate development They have been proposed as therapeutic agents for neurodegenerative disorders and nerve injury However, clinical applications have met with very disappointing results, in part due to difficulties of delivery and pharmacokinetics in the nervous system and unanticipated side effects We have found a way to use small molecule ligands of G protein-coupled receptors (GPCR) to activate Trk receptors in the absence of neurotrophin binding These small molecules keep neurons alive by stimulating the actions of trophic factor receptors Ligands for G protein-coupled receptors represent a novel way of stimulating neurotrophin receptor signaling, however, the mechanism of this process is unknown This grant will investigate the cell biological mechanisms that account for transactivation of neurotrophin receptors in neurons and define the contribution of receptor trafficking and transport to this process Defining the proteins that regulate neurotrophin receptor internalization, translocation and signaling is critical to our understanding of normal neuronal development and function as well as perturbations that occur in response to injury or disease. Our findings are directly relevant to the understanding and treatment of neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases and amyotrophic lateral sclerosis.-

Principal Investigator: CHEN, JUN
Grant Number: 5R01NS044178-02
Title: Apoptosis Execution Pathways in Dopaminergic Cell Death

Abstract: Parkinson's disease (PD) is characterized by progressive and selective loss of dopaminergic neurons in substantial nigra pars compacta. Although the etiology remains unknown, there is considerable evidence that supports the view that programmed cell death (PCD) contributes, at least in part, to the degeneration of dopaminergic neurons in PD. Recent studies have identified a group of terminal caspases, particularly caspase-3/-7, as the central executive molecules in neuronal PCD. Caspase-3 actively participates in dopaminergic neuronal cell death in response to PD-relevant insults, although how caspase-3 is activated in this process is largely elusive. Furthermore, caspase-3 and other terminal caspases may not be the only executioners of neuronal PCD. A novel pro-apoptotic molecule, designated as AIF (apoptosis-inducing factor), has now been identified. AIF, which is activated and released from the mitochondria upon receiving death signals, and potently promotes high-molecular-weight DNA fragmentation and nuclear apoptosis. Bcl-2 family proteins are important PCD regulators and have been implicated in dopaminergic neuronal cell death. The pro-apoptosis Bcl-2 family member Bax is a well-characterized cell death effector, which, upon activation, targets mitochondria and triggers the cytochrome c-dependent intrinsic pathway. However, whether Bax activates AIF and the mechanism by which Bax is activated in dopaminergic neuronal apoptosis is unknown. We have now obtained exciting preliminary data which suggest that 1) caspase-3 activation in dopaminergic neurons is dependent on the intrinsic pathway; 2) both AIF-dependent and caspase-dependent mechanisms may contribute independently and synergistically to the final execution of dopaminergic neuronal apoptosis; 3) Bax is a direct mediator of AIF release in neurons, and the activation of Bax during dopaminergic cell death appears to be p53-dependent; and 4) Bak may be an important cofactor that enhances the pro-apoptotic effect of Bax at the mitochondria. Therefore, the objective of this project is to determine the role of caspase-3-dependent and AIF-dependent death execution pathways in dopaminergic neuronal cell death following PD-relevant insults and to determine the role of Bax in triggering the activation of these two pathways. The proposed studies will be performed using complementary in vitro and in vivo model systems, and will take advantage of our recent cloning of novel dominant-negative inhibitory mutants of caspase-9, Apaf-1 and AIF, and the availability of Bax-deficient mice. The following specific aims will be addressed: 1. Test the hypothesis that the caspase-9/Apaf-1 intrinsic pathway plays a central role in

Principal Investigator: Corcos, Daniel M
Grant Number: 5R01NS028127-10
Title: MOTOR DEFICITS - EXPERIMENTAL AND CLINICAL CORRELATES

Abstract: Parkinson's disease is a progressive neurological disease that dramatically alters the ability of individuals to move. The long-term objective of the proposed research program continues to be to understand how Parkinson's disease changes the way muscles are adapted to perform movements. Aim 1 will test the hypothesis that Parkinson's disease causes an inability to: 1) turn off muscles that are activated, 2) prolong muscle activation and delay antagonist muscle activation for longer movements and, 3) use appropriate patterns of muscle activation to adapt to unexpected changes in load. Aim 2 will determine the extent to which the imbalance between flexor and extensor muscles impairs motor function. The hypothesis is that the electromyographic (EMG) abnormalities address in Aim 1 will be greater in extensor muscles than in flexor muscles. Aim 3 will determine whether disease severity influences the patterns of muscle activation observed in patients with Parkinson's disease. The hypothesis is that disease severity does influence patterns of muscle activation. A second hypothesis is that certain EMG deficits will appear successively in all subjects, while others will appear in a different order in different subjects related to the particular manifestation of the disease. For example, the Principal Investigator expects to see a loss of agonist EMG burst during modulation followed by a decrease in latency of the first antagonist EMG burst. In contrast, the difficulty in turning off muscle activation may not emerge in a fixed relation to the loss of agonist during modulation. Studying two groups of patients who have different degrees of disease severity will test both these hypotheses. The experiments have been carefully chosen to build upon prior studies of muscle activation patterns in neurologically normal individuals, and in patients with Parkinson's disease. Aims 1 and 2 will provide information that can be used to develop models of motor control that apply to a wide variety of movements in Parkinson's disease. Aim 3 will allow the investigators to determine the extent to which EMG and performance deficits manifest themselves at all stages of the disease. Understanding how much muscle activation patterns change to perform movements is important in evaluating pharmacological, neurological, as well as physical therapy interventions that are designed to facilitate movement in Parkinson's disease. -

Principal Investigator: CREUTZ, LELA M
Grant Number: 5F31NS046215-02
Title: Hormone Receptor Actions on Midbrain Dopamine Pathways

Abstract: This proposal will evaluate roles of intracellular estrogen and androgen receptors in the differential hormone modulation of identified midbrain dopamine (DA) systems. Sex differences in the epidemiology of schizophrenia and Parkinson's disease, disorders with underlying DA pathology, as well as studies of hormone manipulations in rats and other mammals suggest that the mesostriatal and mesolimbic DA systems respond differently to androgen and estrogen stimulation. Although some effects may occur independently of intracellular receptors, these studies build on existing data indicating that midbrain DA systems are also subject to intracellular estrogen and androgen receptor-mediated actions. This proposal posits that these influences are anatomically segregated and differentially poised to influence DA neurons in mesostriatal verses mesolimhic pathways. To test this hypothesis, experiments will combine methods of tract-tracing, and single- double- and triple-label immunocytochemistry to localize hormone receptors to specific pathways, and will then use hormone manipulations to test the response of these pathways to receptor-mediated estrogen and androgen stimulation. These studies will concretely place hormone receptive machinery and endpoints of their stimulation in identified, non-endocrine DA pathways, and could identify novel and important neural substrates for the independent modulation of functionally and physiologically distinct DA systems that are differentially important to sensorimotor and cognitive function. -

Principal Investigator: Cronin-Golomb, ALICE M.
Grant Number: 5R21NS043730-02
Title: Optic Flow and Spatial Navigation in Parkinson's Disease

Abstract: Parkinson's disease (PD) is a common age-related neurodegenerative disorder in which multiple aspects of visuospatial cognitive function are impaired, including spatial navigation. Deficits in spatial navigation arise from pathological changes in high-order association areas of the brain but also from defective input from lower-level visual processing areas, including those that mediate optic flow. Optic flow refers to the radial visual patterns that indicate a person's direction of self-movement and maintain gait and postural integrity. The status of optic flow perception in PD is unknown, as is its relation to PD deficits in spatial navigation, such as the inability to maintain a straight path while walking. We propose to examine optic flow perception in PD and its relation to spatial navigation. A critical focus is on the side of onset of motor impairment, contralateral to the hemisphere with predominant basal ganglia dysfunction. PD usually has unilateral onset, and many visuospatial abnormalities arise from right-hemisphere dysfunction. Our preliminary studies suggest that PD patients with left motor onset experience spatial compression of the left visual hemifield, which affects their ability to understand spatial relations and to use spatial information for navigation. We predict that deficits in optic flow perception underlie this perceived spatial compression. Specifically, hemifield differences in optic flow velocities lead patients to misperceive a straight path as curved toward the compressed side of space, and they "correct" their trajectory by walking a curved path. We propose to investigate optic flow and spatial navigation in 18 patients with left-onset PD, 18 with right-onset PD, 18 healthy elderly adults, and 18 healthy young adult adults. Our specific aims are: (1) To manipulate the speed of optic flow in the two visual hemifields and measure each participant's perception of relative speed, which is associated with perceived direction of self-motion. (2) To relate optic flow to spatial navigation. We will use a head-mounted system that provides virtual visual input and records the orientation and spatial position of the participant while walking through veridical space. (3) To relate optic flow and spatial navigation to daily function, as assessed with questionnaires. Our project is conceptually innovative in proposing optic flow deficiencies as a cause of problems of spatial navigation in PD and will generate pilot data in this potentially important area. We have forged novel collaborations between experts in visual psychophysics, behavioral neuroscience, biomedical engineering, and physical therapy to accomplish the goals of this project.-

Principal Investigator: DAUER, WILLIAM T
Grant Number: 1K02NS045798-01A1
Title: The mechanism of MPTP resistance in synuclein null mice.

Abstract: My long-held career goal is to investigate questions of importance to both patient care and fundamental biology. During medical training, I developed a strong interest the basic pathogenic mechanisms of Parkinson's disease (PD), an illness characterized by degeneration of substantia nigra dopamine (DA) neurons and cytoplasmic aggregates of alpha-synuclein (SYN). I came to appreciate the power of genetically modified animals as tools to explore basic aspects of disease pathogenesis, and developed expertise in the generation of such animals. However, I now need to acquire skills necessary to assess the consequences of PD-related mutations on cellular and behavioral aspects of dopaminergic function in these animals. To accomplish this goal, I have developed collaborations with experts in PD research, and will pursue the proposed work within the integrated PD research group at Columbia University. Rarely, PD may be caused by missense mutations in SYN. However, normal SYN function and the mechanism by which pathogenic mutations disrupt SYN biology and lead to PD are poorly understood. MPTP-induced degeneration of DA neurons is a commonly studied model of PD. We find that SYN null mice display striking resistance to MPTP-induced degeneration of DA neurons, and this resistance appears to result from an inability of the toxin to access and inhibit its target, mitochondrial complex I. The goal of this research plan is to exploit this robust phenotype of SYN null mice to gain insight into the normal function of SYN, and explore how this function is altered by PD-causing mutations. In Aim 1 we will measure whether known concomitants of complex I inhibition (increased lactate and reactive oxygen species; decreased ATP) are also impaired in SYN null mice, and characterize processes that control access of the toxin to complex I (vesicular and monoamine transporter function). In Aim 2 we will further explore whether altered synaptic function underlies the MTPT resistance of SYN null mice by testing whether they are selectively resistant to toxins that traffic through the synapse. In Aim 3, by restoring wild type or mutant SYN to specific neuronal populations of SYN null mice, we will test whether the MPTP resistance is a cell autonomous phenomenon and whether pathogenic SYN mutations modify an aspect of its function involved in effecting MPTP-induced neurodegeneration. This proposal exemplifies the type of clinically related fundamental neurobiological research I plan to pursue during my career.-

Principal Investigator: DEBBURMAN, SHUBHIK
Grant Number: 1R15NS048508-01
Title: Yeast Model for Two Neurodegeneration-Linked Proteins

Abstract: Budding Yeast (S. cerevisiae) has emerged as a powerful model system for understanding molecular aspects of many human diseases. Protein misfolding linked to certain neurodegenerative diseases (NDDs) like Huntington Disease, Lou Gehrig's disease, and prion diseases have been successfully recapitulated in S. cerevisiae and led to identification of therapeutically relevant regulators of misfolding. No S. cerevisiae models for Parkinson's Disease (PD) or dentatorubral pallidoluysian atrophy (DRPLA) have been reported. PD is one of the most common NDDs, while DRPLA is a rare inherited NDD of the triplet repeat disease family. In both diseases, misfolding of a specific protein (alpha-synuclein for PD and atrophin for DRPLA) is thought to cause selective neuronal death. Unlike the well-characterized huntingtin protein in Huntington Disease (which shares many similarities to DRPLA), less is known about the misfolding of mutant atrophin in DRPLA. A S. cerevisiae expression system for studying alpha-synuclein has recently been developed in our lab. Preliminary evidence supports that both wildtype and disease-associated mutants are aggregating within yeast cells and upon purification. A similar effort to establish atrophin-1 expression in yeast is underway. To extend initial observations with alpha-synuclein in yeast and fully develop a yeast model for atrophin, three goals are proposed. 1) Misfolding properties between wildtype and mutant versions of both proteins will be investigated in vivo (immunofluorescence and GFP-based localization and assessment of protein half-life) and in vitro (by measuring protease sensitivity and differential solubility). 2) Influences of chaperones and ubiquitin-proteasomal pathway proteins on folding and degradation of these proteins will be assessed in strains compromised for chaperone/proteasomal function, or those that overexpress chaperones, and by co-immunoprecipitation assessment. 3) A fission yeast (S. pombe) expression model for alpha-synuclein and atrophin properties (as in Aim 1) will be developed and compared with the S. cerevisiae model; NDD models have not been reported in S. pombe. These studies may further clarify the molecular bases for misfolding and degradation of PD- and DRPLA-linked proteins and extend the usefulness of yeast models. Importantly, the scientific training of many undergraduates will be supported, strengthening their cell biology and molecular genetics skills and appreciation for model organisms. -

Principal Investigator: DEUTCH, ARIEL Y
Grant Number: 5P01NS044282-03
Title: Dendritic Plasticity in Parkinson's Disease

Abstract: A decrease in striatal dopamine (DA) concentration underlies Parkinson's disease (PD). DA terminals synapse onto striatal medium spiny neurons (MSNs), forming a triad with corticostriatal glutamatergic synapses; the excitatory cortical input is typically onto the head of the dendritic spine and the DA synapse onto the spine neck. DA is thereby critically positioned to gate excitatory glutamatergic inputs to MSNs. A variety of compensatory mechanisms are set into play by decreased striatal DA levels and attempt to maintain normal function in the face of progressive DA loss. Certain changes may afford some benefit but may ultimately be counterproductive. 6-hydroxydopamine (6-OHDA) lesions of the striatal DA innervation result in decreased dendritic spine density and decreased dendritic length in MSNs; a similar picture has been reported in PD. Glutamate has been shown to regulate spine formation and maintenance through NMDA and AMPA receptors, respectively. We hypothesize that striatal DA depletion results in decreased dendritics spine density by increasing glutamatergic transmission, which causes an increase in intracellular calcium levels. The increase in [Ca2+]i results in spine shortening and loss, thus delimiting excitatory drive onto the MSN. However, these dendritic changes may also limit the effectiveness of DA replacement treatment through loss of dendritic spines, on which DA receptors reside. This programmatic effort will test this hypothesis through four projects. The first project will determine if loss of DA tone at the D2 receptor is responsible for the dendritic changes in MSNs, and test the hypothesis that calcium influx through L-type calcium channels is an effector. The second project examines the dopaminergic regulation of CaMKII in the MSN; this dendritically-transcribed Ca2+-dependant enzyme regulates phosphorylation of the GluRl subunit of the AMPA receptor and 2B subunit of the NMDA receptor, and is thus a key to effectiveness of excitatoty glutamatergic transmission. The third project tests the hypothesis that calcineurin (PP2b), a Ca2+- activated phosphatase recruited by dopamine signaling through the D2 receptor, regulates glutamatergic drive onto MSNs and will determine if the decrease in spine density in MSNs is altered by genetic up- or down-regulation of PP2b. The final project will determine changes in dendritic morphology at both the light and electron microscopic level in postmortem material from PD patients and correlate dendritic changes with clinical status; this work will also determine the forms of synaptic reorganization that accompany spine loss. These projects should shed light on the pathophysiology of PD and may lead to development of new strategies aimed at slowing or

Principal Investigator: DUNAH, ANTHONE W
Grant Number: 1K01NS049006-01
Title: REGULATION OF NMDA RECEPTOR TRAFFICKING BY DOPAMINE

Abstract: This grant is a request for a NINDS Career Development Award for Minority Scholars in Neuroscience (K01) to investigate the Regulation of NMDA Receptor Trafficking by Dopamine. Interactions between the dopaminergic and glutamatergic systems in the striatum have implications for the pathogenesis and treatment of Parkinson's disease. My previous work has revealed significant modifications in the properties of striatal NMDA glutamate receptors in animal models of Parkinson's disease. Intriguingly, the alterations in striatal NMDA receptors occur at the level of assembly, phosphorylation and synaptic localization of the subunit proteins, and involved redistribution of receptors between sub-cellular compartments. Furthermore, we recently reported evidence for a rapid dopamine D1 receptor dependent mechanism for the trafficking of striatal NMDA receptors from intracellular compartments to the post-synaptic membrane. The molecular mechanisms for the dopamine D1 receptor mediated sub-cellular trafficking of NMDA receptors in the striatum remain largely unknown. Therefore, I will apply my molecular neuroscience and neuropharmacology backgrounds to experimentally explore and unravel the dopamine receptor dependent molecular mechanisms and signaling pathways underlying the trafficking of striatal NMDA glutamate receptors to brain synapses in primary cell culture system. As a research fellow, I have gained knowledge and received proper training in molecular mechanisms of dopamine and glutamate mediated signal transduction pathways in both in vivo and in vitro systems. The proposed career development program will further my understanding of how the dopamine and glutamate systems in the striatum interact and lead to the pathogenesis of Parkinson's disease. This career development program along with my assembled team of scientists will continue to contribute to my professional and intellectual growth, and eventually establish myself as an independent investigator. The findings from this research proposal may ultimately lead to the development of new therapeutic options for human Parkinson's disease.-

Principal Investigator: ESKANDAR, EMAD N
Grant Number: 2K08NS041851-04
Title: Neostriatal Visual Processing & Initiation of Movement

Abstract: The basal ganglia are a group of subcortical nuclei that are important for motivation and motor control. Disorders of the basal ganglia lead to a variety of disabling movement disorders, the most common of which is Parkinson's disease. The input nuclei of the basal ganglia in primates include the caudate and putamen. The output nuclei include the Gpi and the substantia nigra pars reticulata. Other important nuclei include the substantia nigra pars compacta and the subthalamic nucleus. The input and output nuclei of the basal ganglia are joined by two distinct sets of connections, known as the "direct" and "indirect" pathways. The current model of basal ganglia function holds that the two pathways are in functional opposition and that activation of the direct pathway facilitates movement while activation of the indirect pathway inhibits movement. This explanation works well in empirically explaining what areas of the two pathways are overactive in movement disorders. For example, the Gpi and STN are overactive in PD and hence are effective targets for treatment. However, the nature of the interaction between the two pathways is poorly understood. Most recent models of the basal ganglia emphasize their role in suppressing unwanted movements although this has never been directly tested. Therefore, the primary goal of this research is to understand the role of the basal ganglia in suppressing unwanted movements by recording the activity of basal ganglia neurons in awake behaving primates trained in a movement suppression task. The second goal is to compare the data obtained in primate studies with information obtained by recording from the subthalamic nucleus and globus pallidus of patients undergoing surgery for the treatment of Parkinson disease. In this fashion we hope to understand the derangements of basal ganglia function which occur in PD and to devise better treatment strategies. This work will be conducted in the Department of Neurobiology at Harvard Medical School and in the Department of Neurosurgery at Massachusetts General Hospital.-

Principal Investigator: FETZ, EBERHARD E
Grant Number: 3R01NS012542-29S1
Title: NEURAL CONTROL OF MUSCLE ACTIVITY

Abstract: Unavailable

Principal Investigator: FETZ, EBERHARD E
Grant Number: 5R01NS012542-30
Title: NEURAL CONTROL OF MUSCLE ACTIVITY

Abstract: We plan to investigate the neural mechanisms controlling voluntary hand and arm movement in primates. The functional roles of premotor (PreM) cells in motor cortex and spinal cord will be directly compared. PreM cells with a correlational linkage to forelimb motoneurons will be identified by post-spike effects in spike-triggered averages of EMG activity. The activity of PreM cells and multiple muscles will be documented during multidirectional wrist movements. Monkeys will operate a multi-jointed manipulandum that will allow wrist movements in three directions: flexion-extension, radial-ulnar deviation and pronation-supination. In addition a grip handle will transduce force during a power grip. This repertoire of movements will activate muscles in different synergistic combinations and resolve whether PreM cells and non-PreM cells are organized primarily in terms of muscles or movement parameters. The directional tuning of forearm muscles will be compared with the tuning curves of PreM cells and non-PreM cells. We anticipate finding functionally significant differences between motor cortex cells and spinal interneurons with regard to their relation to muscles and movements. Spinal cord interneurons have been studied largely in immobilized animals; our study will provide new information about the involvement of interneurons in preparation and execution of voluntary movements. These interneurons will be identified by their synaptic inputs from different forelimb muscles and from functionally identified cortical sites. We will also systematically map the movements of arm and hand evoked by electrical stimulation of spinal cord sites; the modulations of these responses during an instructed delay task will reveal the interaction of intraspinally evoked responses with preparation and execution of voluntary movements. Activity of dorsal root afferent fibers also will be recorded during an instructed delay task to document the afferent input to the central nervous system during movement. The axonal excitability of afferent fibers will be tested to investigate task-related modulation of presynaptic inhibition. These studies of the primate motor system will provide unique information essential to understanding and effectively treating clinical motor disorders, like cerebral palsy, stroke and spinal cord injury. -

Principal Investigator: FILOTEO, J V
Grant Number: 5R01NS041372-03
Title: Striatal Contributions to Category Learning

Abstract: Previous studies indicate that patients with damage to the striatum, such as patients with Parkinson's disease (PD) or Huntington's disease (HD), are impaired in certain categorization tasks, but show no impairment in other categorization tasks. These studies suggest that the striatum may be involved in category learning under some circumstances but not others. One possible role of the striatum in category learning is that these structures are involved in learning nonverbal rules, but only when learning is based on corrective feedback under supervised learning conditions. Such a hypothesis is consistent with current models of striatal functioning. However, it is difficult to draw strong conclusions regarding the proposed role of the striatum based on past work because most of these studies used very different categorization tasks that vary along a number of important dimensions. The proposed research remedies these problems by conducting highly systematic studies of category learning in patients with PD. Factors known to impact the verbalizability of categorization rules will be explored, including (1) whether the rule is linear or nonlinear, (2) whether the rule requires information integration across dimensions or selective attention, and (3) whether the stimulus dimensions are separable or integral. In addition, the nature of training (corrective feedback or observation) will also be examined. These factors likely determine the extent to which the striatum is involved in category learning. Each of these factors will be explored within the framework of a highly successful categorization paradigm that has been used extensively in studies of normal cognition, and recently has been extended to some patient populations and normal aging. The paradigm, called the perceptual categorization task, is rigid enough that strong controls can be placed on factors that vary widely across other tasks, but is flexible enough that each of the factors outlined above can be studied in isolation. Further, quantitative models will be applied to the data of PD patients and controls in order to determine more precisely the nature of any observed category learning deficits in the PD patients. -

Principal Investigator: Friedmann, Theodore
Grant Number: 5R01NS044544-02
Title: GENETIC ABBERATIONS IN HPRT DEFICIENCY

Abstract: Lesch Nyhan disease (LND) is a complex neurobehavioral disease caused by deficiency of the X-linked purine salvage pathway enzyme hypoxanthine guanine phosphoribosyl transferase (HPRT). The abnormal neurological phenotype includes retardation, choreoathetosis and self-injurious behavior. The CNS defects are associated with a basal ganglia deficiency of dopamine (DA). A mouse HPRT knockout model displays a relatively normal neurological phenotype but also shows a deficiency of dopamine in the striatum. Primary cultures of midbrain neurons from HPRT-deficient mice demonstrate a reduction of dopamine levels and dopamine uptake. However, to date there has been relatively little progress toward an understanding of the mechanisms by which HPRT deficiency leads to dopamine deficiency. To identify the potential intermediary role of secondary genes functionally downstream of HPRT activity, we have used microarray gene expression analysis on commercially available MU74 oligonucleotide mouse genome chips that interrogate approximately 12,000 known genes and ESTs. In preliminary comparisons of gene expression in dissected striata from wild type and HPRT-deficient mice, we have detected reproducible changes in the expression of a small number of genes and ESTs, including those encoding translation initiation factors IF2s3 and IF3s1, genes associated with striatal dopaminergic neuron function such as sepiapterin reductase that regulates expression of the tetrahydrobiopterin co-factor of tyrosine hydroxylase, and casein kinase I-epsilon that phosphorylates DARPP-32, the principal striatal target for dopamine function. We have also found preliminary evidence for dysregulation of a number of other cDNAs and ESTs of still uncertain relevance to HPRT deficiency. We propose now to complete a more thorough genome characterization of normal and HPRT-deficient mice, to examine the functional effects of aberrant expression of these genes in cultured midbrain and striatal DA neurons and in transgenic and knockout mice. We also plan to determine the biochemical and neurotransmitter effects of genetic correction of these functions by gene transfer techniques. -

Principal Investigator: GIBSON, ALAN R
Grant Number: 3R01NS044592-01A2S1
Title: Influence of the Basal Ganglia on Cerebellar Action

Abstract: Unavailable

Principal Investigator: GIBSON, ALAN R
Grant Number: 1R01NS044592-01A2
Title: Influence of the Basal Ganglia on Cerebellar Action

Abstract: Diseases affecting the basal ganglia produce a variety of movement deficits, and these deficits are often totally disabling. Parkinson's disease, which affects about 1.5 million Americans, is a basal gangliar disease that leads to tremor, decreased spontaneous movement and slowness of voluntary movement. Drug treatment of Parkinson's disease with L-DOPA is only partially effective in relieving the motor symptoms of the disease, and prolonged drug treatment leads to severe side effects such as uncontrollable involuntary movements. Deep brain stimulation at specific sites in the basal ganglia can provide effective relief of Parkinson symptoms. Neither drug treatment nor deep brain stimulation restores damaged neural circuitry in the basal ganglia. Therefore, it is likely that these therapies prevent abnormal basal gangliar output from disrupting processing in other structures related to movement control. One major neural structure related to movement control is the cerebellum, but there are no direct connections between the cerebellum and the basal ganglia. We have discovered that disrupting activity in the cat red nucleus, which connects cerebellar output to the spinal cord, can produce motor symptoms that are strikingly similar to those of Parkinson's disease. The general hypothesis underlying this proposal is that motor deficits produced by basal gangliar disease are mediated by pathways that allow basal gangliar output to disturb processing in structures related to the cerebellum. Specifically, we hypothesize that basal gangliar output from the cat entopeduncular nucleus affects activity of cells in zona incerta, which affects activity of cells in the red nucleus. Our experiments will: 1. Identify regions in the related nuclei that contain cells related to forelimb movement. 2. Determine how these forelimb regions affect movement with activation and inactivation by injection of receptor antagonists. 3. Develop an acute and chronic cat model of basal gangliar disease to test critical aspects of the hypothesis. 4. Identify additional brainstem pathways that allow basal gangliar output to influence cerebellar circuits. The results will provide a deeper understanding of how the basal ganglia and cerebellum interact to control limb movements and will lead to new approaches for the treatment of movement disorders.-

Principal Investigator: GRAYBIEL, ANN M
Grant Number: 5R01NS025529-17
Title: EXTRAPYRAMIDAL SYSTEMS

Abstract: Many researchers are studying the decision and executive functions of cortical areas and cortical networks and the plasticity of these networks. A major question about such cortical processing is how it is influenced by major subcortical systems interconnected with these cortical areas. For the basal ganglia, views have ranged from the basal ganglia serving mainly an automating function to the basal ganglia serving an instructive function, to the basal ganglia being involved both in selection and chunking functions leading to efficient release of behaviors in particular contexts. To approach this issue experimentally, we propose to record simultaneously the activity of neurons in cortex and striatum. We propose to compare and contrast anatomically-defined striosome-based and matrix-based corticostriatal circuits. To do this we will record in cortical areas of the anterior cingulate gyrus and prefrontal cortex, and at the same time record from projection neurons and interneurons in the anatomically defined corresponding corticorecipient zones of the striatum. Our goal is to identify activity patterns in these corresponding cortical and striatal zones in relation to action selection, reinforcement contingencies, decision variables, and automatization. To this end we have developed a joystick task and propose to record simultaneously from multiple sites in cortical areas and the striatum. We propose to analyze ensemble recording data, analyzing variations in responses over time during and between task trials. We have developed a step- by-step experimental strategy to do all ensemble recordings in relation to anatomically-defined corticostriatal and cortricocortical networks originating in cortical regions implicated in behavioral selection, and to identify networks differentially targeting striosomes and matrisomes in the striatum. The proposed work will contribute to a systematic, population-level analysis of cortico-basal ganglia activity related to action selection. This work has potential significance for studies of cortical and basal ganglia function and also for studies related to cortico-basal ganglia loop disorders including neurological disorders such as Parkinson's disease, Huntington's disease and neuropsychiatric disorders such as obsessive-compulsive disorders and Tourette's syndrome.-

Principal Investigator: GROSS, ROBERT E
Grant Number: 1K08NS046322-01A1
Title: Axon Guidance Molecules in Nigrostriatal Regeneration

Abstract: We are interested in developing strategies for the reconstitution of the dopaminergic (DA) nigrostriatal (NS) pathway that degenerates in Parkinson's disease, an important goal because of the inadequacy of current long-term treatments. Attempts to reconstruct this pathway through transplantation of precursor cells or neurons into the nigra of the adult fail, likely as a result of 1) the presence of inhibitory molecules and/or 2) the absence of trophic and guidance molecules in the adult CNS. Here we propose that an understanding of the molecular events that regulate the development of the nigrostriatal pathway will provide insights for strategies designed to improve NS pathway regeneration in the adult milieu. We propose - and have exciting preliminary data to support - that axon guidance molecules (AGMs), important molecules that direct the development of other projection pathways in the CNS, are expressed in the developing DA NS pathway. A series of experiments are proposed to elucidate the role played by AGMs and their receptors in the development of the NS pathway. Our specific aims are to: 1) Define those AGMs whose receptors are expressed in the developing axons of nigral DA neurons; 2) Define the expression of AGM ligands in relation to the developing NS pathway; 3) For those AGMs that are expressed in an appropriate anatomical relationship to influence NS development, and whose receptors are expressed in developing DA neurons, directly demonstrate chemotropic effects on fetal nigral DA neurons in vitro, and their importance in the development of the NS pathway with blocking studies ex vivo. The outcome of the experiments outlined in this proposal will hopefully be the refinement of means to counteract the inhibitory milieu of the adult injured nervous system, and recapitulate the attractive and repulsive factors that direct axonal outgrowth during development, thereby paving the way for novel reconstructive and regenerative strategies to ameliorate the symptoms of Parkinson's disease. The insights derived from these studies may also have applicability in other neurodegenerative diseases, brain injury and stroke. The research outlined is part of a customized five-year plan of training and career development for the Principal Investigator. The proposal includes active mentoring by experienced scientists, access to diverse resources, and an environment uniquely suited to help the PI develop as an independent neurosurgeon-neuroscientist. -

Principal Investigator: GUO, SU
Grant Number: 5R01NS042626-02
Title: Development of Dopaminergic Neurons in Zebrafish

Abstract: Dopaminergic (DA) neurons synthesize and release neurotransmitters dopamine. The importance of DA neurons is underscored by their involvement in multiple human neurological disorders, for instance, Parkinson's disease. Despite their functional significance, the mechanisms determining the development of these neurons are not well understood. Elucidation of these mechanisms is essential to defining and interpreting the causes of disorders affecting DA neurons and developing regenerative therapy for treating Parkinson's disease. Meanwhile, understanding the development of DA neurons will also shed light on fundamental mechanisms governing cell identity and diversity and neural circuit formation in the vertebrate nervous system. The long-term goal of this project is to understand the molecular mechanisms that control the identity and connectivity of subtypes of DA neurons in vertebrates. We are taking a genetic approach in zebrafish, a vertebrate model organism that offers a unique combination of excellent genetics and embryology. We have localized major DA neuronal subtypes in developing zebrafish. By carrying out a genetic screen based on immunohistochemistry, we have identified mutations in three genes that are required for proper development of subtypes of DA neurons. Molecular cloning of the foggy gene revealed the importance of regulated transcription elongation in DA neuron development. Thus, we shall explore how this previously under-appreciated mode of gene regulation is involved in DA neuron development. Phenotypic analysis suggested that the motionless and twin-of-motionless mutations disrupt a signal important for DA neuron induction. Therefore, their molecular identity will be determined. By analysis of cloned genes and existing mutations, we will identify essential machinery involved in controlling DA neuron development. These molecules will not only provide important insights into vertebrate neural development, but may also help develop regenerative therapy for treating neurological disorders such as Parkinson's disease. -

Principal Investigator: GUREVICH, EUGENIA V
Grant Number: 5R01NS045117-02
Title: Dopamine Receptor Trafficking in Parkinson's Disease

Abstract: Arrestins (ARR) and G protein-coupled receptor kinases (GRK) participate in homologous desensitization of many G protein-coupled receptors including dopamine receptors. The rate and extent of desensitization is sensitive to the concentration and activity of ARRs and GRKs in the cells. In their turn, the amount and activity of ARRs and GRKs can be modulated by receptor stimulation. Loss of dopamine in Parkinson's disease (PD) causes motor deficits likely related to changes in responsiveness of striatal dopamine receptors. Dopamine replacement therapy with dopamine precursor L-DOPA, although successful at first, eventually leads to motor complications. Molecular mechanisms of motor disturbances in PD and of L-DOPA- induced side effects remain elusive. Adaptations in the signal transduction pathways mediated by dopamine receptors have been implicated an in neural plasticity induced by dopaminergic denervation and L-DOPA. One of the mechanisms by which loss of dopamine or L-DOPA treatment produce behavioral responses may involve modifications in the receptor desensitization machinery. We hypothesize that loss of adequate dopaminergic stimulation in PD and subsequent non-physiological stimulation during L-DOPA therapy lead to distinct alterations in desensitization and trafficking of dopamine receptors, possibly, due to changes in expression of ARRs and/or GRKs. Specifically, loss of dopamine in PD may reduce the concentration of ARRs/GRKs in striatal neurons, thereby leading to dopamine receptor supersensitivity. First specific aim designed to test this hypothesis includes determination of ARR/GRK expression in the striatum of Pd patients and age-matched controls at post-mortem. In the second aim, the ARR/GRK expression will be studied in the rat model of PD following nigrostriatal lesion and L-DOPA treatment. The third aim focuses on feasibility of a novel way to modulate behavioral and molecular consequences of the nigrostriatal lesion and L-DOPA treatment by facilitating or inhibiting receptor desensitization and trafficking. To that end, lentivirus-mediated gene transfer of GRK2 or its inhibitor into the lesioned rat striatum will be used. The data generated by these studies may open a new promising venue of investigation eventually leading to novel strategies for management of PD. Drugs targeting the receptor desensitization machinery may prove particularly useful for prevention or alleviating of L-DOPA-induced motor complications.-

Principal Investigator: Hablitz, John J.
Grant Number: 5R01NS018145-21
Title: Dopamine Modulation of Prefrontal Cortex Excitability

Abstract: The prefrontal cortex (PFC) plays an important role in governing a number of behaviors, including motivation, emotion learning and memory. The PFC receives a dopaminergic projection from the ventral tegmental area (VTA) which has been specifically implicated in cognitive and neuropsychiatric processes. Dopamine (DA) is believed to be an endogenous neuromodulator in the cerebral cortex and to be important for normal brain function. Clinical and experimental studies have also implicated DA in the pathogenesis of a number of neurological and psychiatric disorders, including epilepsy and schizophrenia. The overall goal of this research is to understand the role of DA in the modulation of activity in local neocortical circuits. The cerebral cortex, particularly the prefrontal cortex (PFC), is heavily innervated by dopaminergic afferents, suggesting this system plays a prominent role in regulating neuronal excitability. Despite the wealth of evidence supporting a role for DA in cognition, neuropsychiatric processes and neurological disorders, our knowledge of the function of DA receptors at the circuit and single cell level is incomplete. It is hypothesized that the net effort of DA will be determined by the interaction of changes in excitatory and inhibitory synaptic activity and alterations in intrinsic neuronal excitability. Specifically, it is planned: (1) to determine if DA receptors positively modulate excitatory inputs to layer II/III PFC pyramidal neurons via a mechanism involving Dl receptors, (2) to ascertain if evoked inhibitory postsynaptic currents (IPSCs) are negatively modulated by DA. Studies will determine if this is a presynaptic effect of Dl receptors mediated by activation of PKA and (3) to characterize and compare the postsynaptic effects of DA in pyramidal cells and fast spiking interneurons. The proposed experiments will provide important new information regarding the role of specific DA receptors in the regulation of local cortical circuits. These data will be important not only in understanding normal cortical functioning, but also in understanding the mechanisms underlying abnormal processes such as schizophrenia, epilepsy and Parkinson's disease, relatedto inappropriate DA signaling. -

Principal Investigator: Hallett, Mark
Grant Number: 5Z01NS002669-20
Title: Physiological Analysis Of Voluntary Movement

Abstract: Unavailable

Principal Investigator: HARRIS-WARRICK,
Grant Number: 5R01NS017323-23
Title: Neurotransmitters, Neuromodulators and Motor Systems

Abstract: The monoamines, including dopamine and serotonin, play important roles in the regulation of behavior in all animals. They act by complex modulatory mechanisms to change the intrinsic firing properties of neurons and the strength of synaptic connections. These changes occur within the context of neural networks that coordinate behaviors, leading to adaptive changes in behavioral output. The overall goal of our laboratory is to show how these cellular mechanisms lead to behavioral plasticity by studying how dopamine, serotonin and octopamine reconfigure the 14-neuron pyloric network in the stomatogastric ganglion of the spiny lobster, Panulirus interruptus. This network generates a rhythmic motor pattern whose properties are determined by the neuromodulators that are present. We have shown that the monoamines can each evoke a unique variant of the pyloric rhythm by direct actions on each of the pyloric neurons and synapses. In this grant, we propose to extend our knowledge of the ionic mechanisms by which the amines reconfigure the pyloric network. We will study amine modulation of ionic currents that generate rhythmic bursting and bistable plateau potential activity, using voltage clamp and multiphoton microscopic calcium imaging. We will also determine whether amines modify synaptic transmission by affecting currents that are different from those modulated to alter firing properties. To better relate these molecular effects to normal neuronal firing, we will drive the neuron in voltage clamp with realistic waveforms that mimic the natural oscillations seen during the pyloric rhythm. In the intact animal, the pyloric network is simultaneously affected by multiple neuromodulators, and these may interact in non-linear ways; we will study this non-linear interaction, or metamodulation, with mixtures of neuromodulators. This work will yield new insights into the detailed mechanisms by which a set of neuromodulators reconfigures a neural network. Because neuromodulatory compounds are conserved and membrane currents and synaptic transmission are similar in network neurons ranging from the pyloric circuit to human cortical networks, our results will suggest common principles for generating behavioral plasticity in vertebrates and invertebrates alike. -

Principal Investigator: HERSHEY, TAMARA G
Grant Number: 5K23NS041248-04
Title: Dopaminergic Modulation of Working Memory in PD

Abstract: The applicant is a clinical neuropsychologist with graduate training in neuropsychology and postdoctoral training in neuropharmacology and positron emission tomography (PET). The goal of this career development award is to integrate and advance these two areas of interest to answer questions about the neuropharmacological and neurophysiological basis of cognitive dysfunction in movement disorders such as Parkinson's disease (PD). This award will provide the applicant with training in the technical and theoretical issues related to using cognitive and pharmacological activation techniques in functional magnetic resonance imaging (fMRI). Long-term objectives are to address questions about the neural basis of cognitive dysfunction in movement disorders related to dopaminergic and/or basal ganglia dysfunction, such as PD, Tourette's syndrome and Huntington's disease. In addition, questions about the effects of dopaminergic treatments for these and other disorders (e.g. dystonia) on cognitive and neurophysiological functioning are also of interest. Cognitive dysfunction in these diseases, either due to the disease process itself or its treatments, can be limiting and disabling. Understanding the neurophysiologic basis for these symptoms may aid in assessing the effectiveness of current treatments or in developing better treatments. During the award period, the applicant will develop expertise in the use of fMRI, cognitive and neuropharmacological techniques to study these disorders, and will continue to hone her clinical skills in the neuropsychological assessment of movement disorders. The applicant will apply these new techniques to investigate the role of dopamine in working memory. The specific aims of the proposed studies are to test the hypothesis that 1) PD affects prefrontal cortex involvement in working memory and 2) dopaminergic modulation of working memory primarily occurs due to changes in lateral prefrontal cortical activity. To test these hypotheses, the applicant will first perform a behavioral study examining the effects of a steady-state infusion of levodopa, a dopamine precursor, on verbal and spatial working memory in PD patients and controls. The results of this study will then guide the choices of working memory tasks for an fMRl study. Subjects will be asked to perform working memory tasks before and during a steady-state infusion of levodopa. Modulation of the lateral prefrontal cortex is predicted during levodopa infusion. The degree of modulation is predicted to depend on baseline dopaminergic status (PD vs control) and the degree of memory load (low vs high). -

Principal Investigator: HORVATH, TAMAS L
Grant Number: 5R01NS041725-03
Title: Uncoupling Protein 2 Promotes Neuronal Survival

Abstract: We have identified the existence of mitochondrial uncoupling protein 2 (UCP2) in homeostatic circuits of healthy rodents and non-human primates. We also showed that ectopic expression of this uncoupling protein is induced in different models of neurodegeneration, including models of Parkinson's disease, hypoxia, epilepsy or trauma-induced brain injury. The expression of UCP2 in these experiments was associated with subpopulations of neurons and microglial cells at the site of the degenerative processes and predicted cells with the longest survival after the initial insult. In our preliminary studies, UCP2 overexpressing animals had diminished levels of free radical production in the brain and responded to transection of the entorhinal pathway with suppressed caspase 3 activation. We hypothesize that the induction of UCP2 in neurons and glial cells during pathological neurodegeneration is an attempt to protect and rescue injured neurons. Three Specific Aims are proposed to test this hypothesis: Specific Aim 1 To determine the role of the UCP2 gene product in intracellular calcium homeostasis and protection of cells in vitro by studying PC12 cells and primary cultures of retinal ganglion cells with and without UCP2 transfection and primary cultures of retinal ganglion cells taken from UCP2 transgenic, UCP2 knockout and wild type mice. The effects of oxygen and glucose deprivation and glutamate agonists will be assessed on cell death patterns and intracellular calcium metabolism in these cultures. Specific Aim 2 To determine the pattern of neurodegeneration, mitochondrial uncoupling activity, cytokine and ATP production in the brains of UCP2 knockout mice, UCP2 overexpressing transgenic mice and wild type mice undergoing hypoxia-, seizure- or 1-methyl-4-phenyl- 1,2,5,6 tetrahydropyridine (MPTP)-induced neurodegeneration. Specific Aim 3 To assess the effects on phenotype development of superoxide dismutase 2 knockout animals that are crossbred with either UCP2 knockout or UCP2 overexpressing mice. In these experiments, we will follow the phenotypic alterations by assessing neuronal loss, level of mitochondrial uncoupling activity, cytokine, free radical and ATP production and intracellular calcium levels using morphological, biochemical and molecular biological approaches. The results of the proposed studies will shed light on a novel mitochondrial mechanism that plays critical roles in the suppression of neurodegeneration regardless of the initial cause of disease. This will furnish one common target for the development of drugs against a variety of neurodegenerative pathologies, including those associated with hypoxia, epilepsy, Parkinson's, Alzheimer's and Huntington's Disease. -

Principal Investigator: HSU, SHU C
Grant Number: 5R01NS038892-05
Title: Molecular Mechanisms of Neurite Outgrowth

Abstract: The precise yet dynamic networking among nerve cells is the cellular basis of many if not all brain functions. Alteration or disruption in this network is likely to result in mental deficiencies and/or illnesses. To establish and maintain this neuronal network, neurons adopt a highly specialized and flexible morphology; the formation and modulation of this specialization requires precisely targeted protein/membrane addition to designated plasma membrane domains. A molecule implicated in this targeting process is the Exocyst complex, a macromolecule essential for protein/membrane targeting and critical for neuronal development. Mouse embryos with an Exocyst subunit deletion die upon gastrulation at the onset of neural induction. As a first step to understand the molecular mechanisms of the Exocyst function in neuronal development, we identified the molecular associations of the Exocyst complex and studied its function in neuronal differentiation. We found that the Exocyst complex associates with microtubules and septins, a familty of GTPases whose members have been found to be present in Alzheimer neurofibrillary tangles and act as a substrate for Parkin, a protein implicated in the Parkinson's disease. Septins, in turn, were found to associate with the actin network. Both the Exocyst complex and the septin filament are dynamic molecules which change their subcellular localization upon neuronal differentiation in response to the MAP kinase pathway. In addition, we have also found that the Exocyst complex co-immunoprecipitates with the CDK5 kinase activator p35. We hypothesize that the Exocyst complex coordinates with cytoskeletons, under regulation by signaling molecules such as RalA and the p35/CDK5 kinase system to mediate protein/membrane targeting to designated plasma membrane domains for the generation of neuronal polarity. In this proposal, our objectives are to characterize the Exocyst complex association/coordination with cytoskeletons, to analyze how these interactions contribute to neuronal development and to study the regulation of the Exocyst complex molecular associations and function during neuronal development. These studies aim to further our understanding of the molecular mechanisms and regulations of the Exocyst complex function during neuronal development, and to guide future experimental designs to study the involvement of the Exocyst complex and its associations in neuronal regeneration and degeneration. -

Principal Investigator: HUNG, ALBERT Y
Grant Number: 5K08NS041411-04
Title: Activity-Dependent Regulation of Synapses by Shank

Abstract: The goal of this project is to investigate the role of a newly discovered postsynaptic protein, Shank, in the regulation of dendritic spine morphology and cytoskeleton. Local electrical stimulation induces growth of dendritic spines, suggesting that synaptic activity directly modulates neuronal architecture and circuitry. The molecular basis for these activitydependent changes is not known, but probably involves postsynaptic proteins that interact with receptors and/or cytoskeletal elements. Shank acts as a putative scaffold for multiple glutamate receptor subtypes and also binds to the actinbinding protein cortactin, which has been implicated in dynamic cytoskeletal rearrangement and translocates to synapses in response to glutamate. This study examines the role of Shank in the regulation of dendritic spines and its in vivo function through three specific aims. First a combination of cell biological, biochemical, and dominant inhibitory approaches will be used to determine the mechanism for glutamateregulated cortactin translocation to synapses, and to identify if Shankcortactin interaction is required for this response. Second, how Shank induces spine growth will be studied by structurefunction analysis. Finally, a genetic approach, generation of a Shank1 "knockout" mouse, will be used to investigate the role of Shank proteins in brain development, in postsynaptic receptor organization, and in learning and memory. The longterm goal of the candidate is to understand how aberrant synaptic transmission contributes to neurologic disease. Synapses are the signal processing units of the brain, and overexcitation of synapses by glutamate is thought to play a role in both acute neuronal injury (such as stroke and seizure) and chronic neurodegenerative conditions (including Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis). Understanding how postsynaptic proteins, such as Shank, regulate activitydependent synaptic plasticity may shed light on mechanisms of glutamate toxicity. The immediate goal is to obtain training in the most uptodate techniques in molecular genetics, protein biochemistry, and cellular neurobiology, sponsored by Dr. Morgan Sheng, which will enable him to become a productive, independent molecular neurologist. -

Principal Investigator: Jacobs, JESSE V
Grant Number: 1F31NS048800-01
Title: rTMS over premotor cortices during stepping in PD

Abstract: This project will explore the role of the Supplementary Motor Area (SMA) and dorsal Premotor cortex (dPMC) in the postural preparation and execution of voluntary stepping with and without visual targets in healthy and Parkinson's disease (PD) human subjects. The excitability of these cortical regions will be 'temporarily depressed by repetitive transcranial magnetic stimulation (rTMS). Measures of step timing and placement, prepatory shifts in foot pressure, and electromyographical records of muscle activity will provide data regarding the effect of PD, visual condition, levodopa, and rTMS on step preparation and execution. Hypotheses: (1.) The SMA is important for movement preparation and postural coordination prior to a voluntary step, and the symptom of bradykinesia (slow step initiation and impaired preparatory shifts in foot pressure) in PD is associated with impaired activity in the SMA. (2.) The dPMC is important for integrating visual input to modify voluntary steps (step placement and trajectory), and PD subjects exhibit an increased dependence on vision and the activity of the dPMC to execute voluntary steps.-

Principal Investigator: JAEGER, DIETER
Grant Number: 5R01NS039852-05
Title: CONTROL OF SPIKING IN BASAL GANGLIA OUTPUT NEURONS

Abstract: The objective of the proposed research is to determine how the activity of neurons in the substantia nigra pars reticulata (SNr), one of the major output nuclei of the basal ganglia, is controlled by synaptic input. Simple network models of basal ganglia function and disorders assume that the activity of output neurons is determined by summing the amount of inhibitory and excitatory inputs received. It is clear, however, that single neurons have active intrinsic mechanisms by which synaptic inputs may be integrated in a highly complex non-linear fashion. These complex properties of synaptic integration will be examined in SNr neurons by combining in vitro whole cell recording, extracellular recording and computational modeling. First the passive and then the active properties of these neurons will be catalogued using whole-cell recordings in rat brain slices. These experiments will use current and voltage-clamping in conjunction with pharmacological blockade of various voltage- and ligand gated channels to isolate and characterize purely passive membrane properties and specific voltage-dependent conductances. Recorded neurons will be intracellularly stained and reconstructed histologically with Neurolucida, and the quantitative morphometric data obtained will be used along with the electrophysiological data to construct a compartmental model of SNr neurons. The model will be adjusted and fine tuned by comparing the behavior of the model to that of SNr neurons in whole cell recordings in vitro and in extracellular single unit recordings in vivo, while constraining the parameters to those obtained in the recording experiments. To study the mechanisms by which synaptic input controls activity, the parameters of synaptic inputs including the time courses and amplitudes of excitatory and inhibitory inputs will be measured and used in the model. Finally, realistic sequences of synaptic input, inferred from in vivo and in vitro recordings of SNr neurons will be input to the model to determine the input-output function of SNr neurons. -

Principal Investigator: JAKOWEC, MICHAEL W
Grant Number: 5R01NS044327-03
Title: Glutamate-dopamine plasticity in nigrostriatal injury

Abstract: The MPTP-lesioned mouse serves as an excellent model to study the mechanisms involved in the return of striatal dopamine after basal ganglia injury. The administration of MPTP to C57BL/6 mice leads to the destruction of nigrostriatal dopaminergic neurons and subsequent depletion of striatal dopamine. An advantage of MPTP-lesioning is that the degree of neuronal cell death can be titrated such that remaining dopaminergic neurons may act as a template for repair and recovery in response to the injury. Our hypothesis is that glutamate, acting through altered expression of the AMPA-subtype of receptor, activates the transcription factor phospho-CREB and leads to increased tyrosine hydroxylase expression and axonal sprouting in surviving nigrostriatal dopaminergic neurons. This research proposal is designed to define changes that take place after MPTP injury in the expression of AMPA receptors (including their phosphorylated state), the transcription factor CREB, dopamine receptors (Dl, D2, and D3), and the growth-associated protein GAP-43. The effect of blocking glutamate neurotransmission with the AMPA receptor antagonist GYKI-52466 on these parameters will be determined. The molecular tools of immunocytochemistry, western immunoblotting, in situ hybridization, and anterograde labeling will be used to define the mechanisms involved in the return of striatal dopamine. The long-term goal of these studies is to elucidate features of plasticity following injury to the brain and to identify new therapeutic interventions for the treatment of neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, and aging. -

Principal Investigator: KALYANARAMAN,
Grant Number: 2R01NS039958-05
Title: Role of Neuronal NOS & Superoxide in Neurodegeneration

Abstract: Long-term goal: The broad objectives of this renewal are to understand the mechanism(s) by which mitochondrial neurotoxins such as 1-methyl-4-phenylpyridinium (MPP+) selectively destroy dopaminergic neurons in the substantia nigra, leading to the development of Parkinson's disease (PD). Reactive oxygen and nitrogen species (ROS/RNS)-mediated damage has been implicated in age-related neurodegenerative diseases like PD. Hypothesis: (i) MPP+ generates mitochondria superoxide (02*) and hydrogen peroxide (H202), and inactivates mitochondrial iron-sulfur-proteins (e.g., aconitase). This stimulates transferrin receptor (TfR)-mediated uptake of iron. (ii) MPP+-induced H202 and iron transported through TfR cause enhanced degradation of tetrahydrobiopterin (BH4), an essential co-factor for neuronal nitric oxide synthase (nNOS), tyrosine hydroxylase (TH), and dihydropteridine reductase (DHPR) activities. BH, depletion causes "uncoupling" of nNOS to form O2* and inactivation of TH and DHPR leading to dopamine depletion. (iii) MPP+-induced O2*, H2O2, and Tf-iron stimulate aggregation of a-synuclein, a neuronal presynaptic protein leading to apoptosis or programmed cell death. Aims: 1.) Investigate the effect of TfR-dependent iron and mitochondrial ROS in neuronal cell apoptosis in response to MPP+. 2.) Assess the modulatory effect of BH4 depletion on nNOS-generated nitric oxide ('NO)/O2* ratio and on BH4-dependent enzyme controlling dopamine synthesis. 3.) Elucidate the role of ROS, Tf-iron and BH4 depletion on a-synuclein aggregation and apoptosis in neuronal cells treated with MPP+. Methods: We will use both dopaminergic and non-dopaminergic cells (neuroblastoma and cerebellar granule neurons). The following redox-parameters will be measured: GSH and lipid peroxides; aconitase, complex-I, and iron-regulatory activities; TfR expression and 55Fe uptake; a-synuclein expression and aggregation; caspase activation and apoptosis. ROS/RNS will be determined by fluorescence and spin-trapping techniques. Significance: PD affects about 1% of population over the age of 50. Emerging data allude to environmental mitochondrial toxins as a causative factor. Novelty: This proposal sheds new light on the synergistic role for MPP*-induced mitochondrial ROS, iron, BH4-induced nNOS uncoupling, dopamine depletion and alpha-synuclein aggregation in neuronal toxicity of PD and other mitochondrial diseases.-

Principal Investigator: KANG, UN Jung
Grant Number: 5R01NS043286-02
Title: The neuroprotective effect of tetrahydrobiopterin

Abstract: While multiple etiologies are likely to account for Parkinson's disease (PD), the core pathogenic feature is degeneration of dopaminergic neurons, particularly those in the substantia nigra pars compacta (SNpc), with shared common final pathways involving oxidative damage, mitochondrial dysfunction, or both. Therefore, one may hypothesize that dopaminergic neurons in the SNpc are selectively vulnerable to oxidative stresses and/or mitochondrial disruption and understanding the mechanism of this selectivity may reveal the pathogenesis. However, our data show that ventral mesencephalic dopaminergic neurons in culture have an enhanced antioxidant capacity, as they are better able to resist oxidative stresses such as glutathione depletion and peroxide treatment than nondopaminergic neurons. In addition, their enhanced antioxidant capacity is reflected in lower reactive oxygen species (ROS) and higher reduced glutathione levels than nondopaminergic neurons. We hypothesize that an enhanced antioxidant capacity is essential for the survival of dopaminergic neurons that may be subjected to increased oxidative stress exerted by dopamine and its metabolites. We postulate that disruption of this innate antioxidant capacity makes them vulnerable to additional environmental insults and thereby leads to selective degeneration. We noted that the enhanced antioxidant capacity in ventral mesencephalic dopaminergic neurons is due to tetrahydrobiopterin (BH4), which is the cofactor for tyrosine hydroxylase, the enzyme producing dopamine, but also lowers superoxide levels, partly be direct scavenging effect and modulates mitochondrial function. First, We will study the effect of BH4 on mitochondrial bioenergetics and function including initiation of death pathways. Second, we will examine the role of BH4 on NO and superoxide generation and in modulating other endogenous antioxidant systems. Third, the neuroprotective function of BH4 against PD models such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, rotenone toxicity, and glutathione depletion will be tested in vivo and in organotypic slice cultures, using hph-1 mice that are deficient in BH4, production.-

Principal Investigator: KAPLITT, MICHAEL G
Grant Number: 1K08NS044978-01A2
Title: PTEN Anti-Oncogene: Neuronal Function and Toxicity

Abstract: The PTEN anti-oncogene is among the most frequently mutated genes in malignant brain tumors. Normally, PTEN is a lipid phosphatase which blocks malignant phenotypes primarily by inhibiting the PI3 Kinase/AKT pathways, but PTEN can also act as a protein phosphatase. PTEN is expressed in brain late in development, and neuronal expression continues throughout adult life. Although loss of PTEN can cause neuronal hyperplasia, little is known about the role of PTEN in neuronal development or in normal neurons. Pathways influenced by PTEN suggest that this anti-oncogene may increase neuronal sensitivity to toxicity and/or degenerative processes, which is supported by our preliminary data. This proposal will first determine whether PTEN can modulate sensitivity of cultured neuron-like cells to toxins used in models of Alzheimer's disease and Parkinson's disease. While studying this hypothesis, we have unexpectedly found that PTEN blocks NGF signaling in PC12 cells, and this appears to be at least partially due to inhibition of expression of trkA and p75 NGF receptors at the protein and mRNA levels. DNA microarray then revealed that PTEN can inhibit expression of several genes, including tyrosine hydroxylase and GTP cyclohydrolase 1. Since this may also have implications for neuronal function and for Parkinson's disease, the second Aim of this proposal will also explore the mechanism by which PTEN inhibits expression of these genes. The final Aim of this proposal will explore the effect of age and neurotoxins used in models of neurodegenerative disorders on PTEN levels and function to determine the biological relevance of data generated from the first two Aims. These studies and my development as an independent clinical scientist will be significantly advanced by Dr. M. Flint Beal, who will serve as my sponsor and who is a leading expert in neuronal degeneration in PD and AD. Additional mentoring by Dr. Eric Holland, a leading expert on anti-oncogene signal transduction, will also add significantly to my scientific growth and will also help me to realize many of the Specific Aims of this proposal. The environment at Cornell and the strong support of my institution will permit me to focus upon these studies with minimal distractions. My scientific background is substantial, and this will facilitate realization of the goal of this project. This plan outlined in this award will, however, enhance previously underserved aspects of my education while focusing on an important scientific question, in order to promote a successful transition to scientific independence.-

Principal Investigator: KATZ, PAUL S
Grant Number: 5R01NS035371-14
Title: INTRISIC NEUROMODULATION OF A SMALL NEURONAL NETWORK

Abstract: Neuromodulation, the neurochemical alteration of neuronal and synaptic properties, is important for motor pattern generation and behavioral plasticity, yet few studies have explored the dynamics of neuromodulatory signaling in neuronal circuits. The goals of this project are to understand how intracellular signals mediating neuromodulatory actions are dynamically integrated over time and how this contributes to the production and plasticity of a motor behavior. The system being studied is the central pattern generator (CPG) underlying the rhythmic escape swimming response of the gastropod mollusc, Tritonia diomedea. This model system is uniquely suited to address these issues because it contains identified neurons, intrinsic to the CPG circuit, that use serotonin (5-HT) to evoke neuromodulatory actions in other CPG neurons. The proposed experiments test the hypotheses that the dynamics of 2nd messenger signaling evoked by this "intrinsic neuromodulation" play a direct role in motor pattern production and that summation of 2nd messenger signals contributes to termination of the behavior and to its habituation. Aim I is to visualize the temporal dynamics of Ca 2+ and cAMP during motor pattern production. Aim 2 is to identify which second messengers mediate particular neuromodulatory actions of 5-HT and serotonergic neurons. Aim 3 is to test the behavioral roles of dynamic biochemical signaling during motor pattern generation and plasticity. The experimental methods include electrophysiological and optical recordings in situ and in primary cell culture. Real-time changes in Ca 2+ and cAMP levels are measured using confocal and multiphoton imaging of fluorescent indicators. Second messengers are manipulated both pharmacologically and in real time with rapid photolysis of caged compounds. The objective is to directly observe and perturb dynamic biochemical signals during the production of the motor behavior. These experiments will elucidate general principles of how neuromodulatory signals are temporally integrated over behaviorally relevant time scales, thereby uniting the operation of neuronal networks with intracellular biochemical signaling networks. Understanding the dynamics of neuromodulatory mechanisms underlying signaling by biogenic amines in a motor system is likely to have significance for diseases related to defects in aminergic signaling such as Parkinson's disease and Huntington's disease.-

Principal Investigator: KEEFE, KRISTEN A
Grant Number: 5R01NS041673-03
Title: Regulation of Striatal Neurons by NMDA Receptor Subtypes

Abstract: Parkinson's Disease (PD) is a devastating movement disorder consequent to massive death of neurons in the substantia nigra. An important functional consequence of this cell death is depletion of the neuromodulator dopamine (DA) within the striatum. In addition to the DA input, the striatum also receives major glutamate input from the cerebral cortex and thalamus. The ameliorative effects of DA agonists in animal models of PD are potentiated by antagonists of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor. In addition, NMDA antagonists block the appearance of dyskinesias in Parkinsonian animals treated with DA agonists and also block DA agonist and antagonist-induced immediate early gene expression in the intact striatum. Recent data from our laboratory indicate that distinct NMDA receptors selectively interact with Dl and D2 DA receptors to regulate immediate early gene expression in striatonigral and striatopallidal efferent neurons in both DA-depleted and intact animals. Furthermore, evidence suggests that corticostriatal and thalamostriatal afferents selectively affect the function of D2 receptor-containing striatopallidal and Dl receptor-containing striatonigral neurons, respectively. Finally, data from studies on other brain regions indicate that different NMDA receptor subtypes can be targeted to synapses associated with distinct afferent pathways within the same neuron. Thus, the goal of this proposal is to test the hypothesis that Dl and D2 dopamine receptors selectively interact with distinct NMDA receptors by virtue of the afferent-selective expression of distinct post-synaptic NMDA receptors in striatal efferent neurons. This hypothesis will be tested by completing the following specific aims: A) Establish how generalized the selective association of specific NMDA and DA receptors is across striatal efferent neuron responses. B) Determine the pharmacology of NMDA receptors mediating corticostriatal and thalamostriatal activation of immediate early gene expression in striatal efferent neurons in vivo and examine the modulation of this activation by DA receptor manipulations. C) determine the kinetics and pharmacology of NMDA receptor-mediated EPSCs evoked in striatal efferent neurons by activation of cortical and thalamic afferents and examine the modulation of those EPSCs by DA receptor manipulations. It is anticipated that the results of these experiments will provide new insight into the functional relationship between DA and NMDA receptors in the regulation of striatal efferent neurons and will lead to important new advances in therapeutic interventions for the treatment of PD and other disorders of the basal ganglia. -

Principal Investigator: KELLY, VALERIE E
Grant Number: 1L30NS049916-01
Title: Subthalamic Nucleus Stimulation in Parkinson's

Abstract: Unavailable

Principal Investigator: KITA, HITOSHI
Grant Number: 5R01NS042762-03
Title: Physiology and Anatomy of the Basal Ganglia

Abstract: We have hypothesized that the external segment of the pallidum (GPe) is located at the center of the basal ganglia connections and plays a key role in the physiology and pathophysiology of the basal ganglia. To understand how the neuronal activity of the GPe is controlled under physiological and pathophysiological conditions, the nature of both the synaptic inputs to the GPe neurons and the properties of the postsynaptic membrane should be fully characterized. Anatomical knowledge as well as theories on the basal ganglia motor control predict that the stimulation of the cerebral cortex would induce powerful disynaptic, through the neostriatdum (Str), inhibition in the GPe. However, a striking observation obtained in monkey unit recording study as well as intracellular recording in anesthetized rats was that the cortical stimulation-induced inhibition in the GPe was weak and was dominated by the disynaptic excitation through the subthalamic nucleus (STN). When the STN was chemically blocked, cortical stimulation induced a long duration powerful inhibition in the GPe. Thus, aim 1 is to test a possibility that glutamatergic inputs suppress GABAergic inhibition in the GPe. The STN blockade also caused slow and strong oscillation of GPe neurons. Aim 2 is to investigate the nature of the slow oscillation. Our experiments in the monkey also suggest that GPe neurons receive tonic excitatory input even after the STN blockade. The possible excitatory sources to the GPe, other than the STN, include the centromedian-parafascicular complex (CM-Pf) and the dorsal raphe nucleus. Aim 3 is to study the anatomical and physiological properties of the CM-Pf inputs to the GPe. Aim 4 is to investigate the effects of 5-HT agonists on the GPe neurons and also on the GABAergic synaptic transmissions in the GPe. Experiments will use whole cell recording method in rat brain slice preparations, unit recording method in awake monkeys and an anterograde neurotracing method in the rat. At the end of the proposed projects, we should be able to offer an anatomical and physiological basis for explaining how the synaptic inputs might control the neuronal activity of the GPe.-

Principal Investigator: KOCHANEK, PATRICK M
Grant Number: 5R01NS038087-06
Title: Adenosine and Traumatic Brain Injury

Abstract: In traumatic brain injury (TBI), adenosine activates high affinity A1 receptors conferring anti-excitotoxic effects. After TBI, however, adenosine levels are high-activating lower affinity A2a receptors that may down-regulate A1 and confer direct neurotoxicity. In models of Parkinson's disease, A2a receptor antagonists are neuroprotective. We reported neuroprotective effects of adenosine after TBI-via anti-excitotoxic effects at the A1 receptor. However, activation of lower affinity A2a receptors could negate this benefit. Out pilot studies show that A2a receptor ko mice are neuroprotected vs wt after experimental TBI and administration of the A2a agonist CGS21680 worsens outcome. However, A2a receptor agonists increase cerebral blood flow (CBF), a finding that must be reconciled. Our clinical studies show that increases in adenosine in cerebrospinal fluid (CSF) are associated with poor outcome. A therapeutic opportunity for A2a receptor antagonists is suggested; however, this pathway must be first studied in experimental TBI. A2a receptor signal transduction is coupled to adenyl cyclase (AC). We reported progressive increases in cAMP levels in CSF after clinical TBI. Hypothesis: Treatment with A2a receptor antagonists or inactivation of the A2a receptor will improve outcome after experimental TBI. Using the controlled cortical impact (CCI) model of TBI in mice and rats, we will address five aims: (1) Determine A2a receptor dynamics after CCI in mice and rats, (2) Assess the role of the A2a receptor in determining biochemical (glutamate, ACh, cAMP), functional, and histological outcome after CCI in mice and rats, including A2a receptor ko mice, (3) Assess the effects of A2a receptor activation on CBF and cerebral metabolic rate after CCI in rats. (4) Define the role of A2a receptor-mediated activation of AC after CCI in mice and rats, (5) Determine the role of the A1 receptor in the detrimental effects of A2a agonists in CCI using A1 receptor ko mice, and (6) To bridge bench and bedside after severe TBI in humans, using CSF samples from 161 patients, we will quantify levels of the non-selective adenosine receptor antagonist caffeine (and metabolites) to test the hypothesis that acute caffeine consumption is associated with favorable outcome and reduced cAMP. These studies explore the most promising adenosine-based therapy for TBI-A2a receptor antagonists. Our bench to bedside track record ensures translation to the clinic. -

Principal Investigator: KONTOPOULOS, EIRENE
Grant Number: 1F31NS049869-01
Title: Mechanisms of Neurotoxicity in Parkinson's Disease

Abstract: Our long-term objective is to elucidate the underlying mechanisms of neuronal death in Parkinson's disease (PD). The identification of several genes exhibiting linkage to PD has not yet led to the understanding of how their protein products bring about cell death. Though in vitro studies have been instrumental in identifying potential mechanisms of neurodegeneration, their findings need to be corroborated in vivo. I propose to utilize Drosophila genetics to investigate putative protein interactions among three PD-linked genes: synphilin-1, alpha-synuclein, and parkin. My primary strategy will be to investigate the inherent toxicity of synphilin-1 and its PD-associated mutation, R621C. Furthermore, genetic interactions between both forms of synphilin and either alpha-synuclein or parkin will be examined. These efforts will culminate in the investigation of genetic interactions among synphilin-1, alpha-synuclein, and parkin. -

Principal Investigator: KOTZBAUER, PAUL T
Grant Number: 1K08NS048924-01
Title: Neurodegenerative consequences of PanK2 mutations

Abstract: The candidate is an M.D./Ph.D neurologist who is currently a trainee in the Center for Neurodegenerative Disease Research. His goal is to develop additional research skills and experience needed to become an independent clinician scientist working to understand the pathogenesis of neurodegenerative diseases. The proposed research project focuses on neurodegeneration with brain iron accumulation (NBIA), which causes progressive impairment of speech, movement and cognition. At the neuropathological level, NBIA is characterized by iron accumulation, inclusion formation, signs of oxidative stress, and death of multiple neuronal populations. These features are also seen to varying degrees in other neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease. Mutations in the gene for pantothenate kinase 2 (PanK2) were recently identified in a subset of NBIA cases. The PanK2 gene encodes an enzyme involved in coenzyme A (CoA) synthesis, a critical pathway linked to a number of cellular processes, including fatty acid synthesis, energy production, and possibly, synthesis of anti-oxidant molecules. The long term objectives of this project are to understand how PanK2 mutations lead to iron accumulation, oxidative stress, inclusion formation, and neuronal death. The proteolytic processing, mitochondrial localization and in vitro catalytic properties will by characterized for mutant Pank2 proteins and compared to the wild type human PanK2 protein. Cell culture systems will be established in which PanK2 expression is eliminated and in which wild type or mutant PanK2 proteins are over-expressed. Mice that lack PanK2 expression will also be generated. Cell lines and mice lacking PanK2 expression will be examined for changes in levels of biochemical intermediates hypothesized to be dependent on PanK2 function. Finally, neuronal and non-neuronal cells lacking PanK2 will be examined for signs of increased oxidative stress, susceptibility to oxidative injury, cellular and mitochondrial import of radio labeled iron, and inclusion formation.-

Principal Investigator: KULAK, JENNIFER M
Grant Number: 5F32NS043079-04
Title: Changes in nicotinic receptors in parkinsonian animals

Abstract: As an initial approach, the research proposed in this application will identify specific subtypes of nicotinic acetyicholine receptor (nAChR) present in the nigrostriatal system which may be investigated for development of novel therapeutics for treatment of Parkinson's disease. Evidence that nAChRs may provide a therapeutic target for treatment include: (i) the demonstration of an inverse relationship between smoking and the incidence of PD; (ii) alleviation of the locomotor symptoms of PD with nAChR agonist administration; (iii) selective changes in expression of nAChR mRNAs in squirrel monkeys lesioned with the nigrostnatal toxin MPTP; and (iv) preliminary results indicating alterations in nAChR binding in parkinsonian non-human primates. Nicotinic receptor subtypes expressed in the nigrostriatal system will be investigated using 1251-epibatidine, 3H-cytisine, and '251-a-bungarotoxin autoradiography and competition with subtype-selective IIAChR ligands in the substantia mgra, caudate, and putamen of control and parkinsonian monkeys and the neurotransmitter makeup to which specific subunits colocalize will be determined by dual label in situ hybridization in the substantia nigra.-

Principal Investigator: LANGSTON, J W
Grant Number: 5R01NS034886-07
Title: MECHANISMS OF DOPA-DYSKINESIAS IN PARKINSONIAN MODELS

Abstract: This is the first competitive continuation of an ongoing NIH application to investigate dopa-dyskinesias in parkinsonian MPTP-lesioned monkeys. The long-term goal of this work is to elucidate the mechanisms underlying this devastating complication of chronic L-dopa therapy, which is a major barrier for the successful treatment of Parkinson's disease. During the course of our ongoing grant, we unexpectedly observed that normal animals also developed dopa-dyskinesias, in contrast to previous work which suggested that a nigrostriatal deficit is essential for this complication of L-dopa therapy. This new non-lesioned model of dopa-dyskinesias may provide insight concerning the etiology of these movement abnormalities because it allows us to investigate this phenomenon in a setting that is not confounded by an already damaged nigrostriatal system. By examining the biochemical changes caused by L-dopa in unlesioned as compared to MPTP-lesioned animals, we should be able to identify common molecular mechanisms that underlie the development of typical dyskinesias. In this competitive continuation, the behavioral, cellular and molecular mechanisms associated with L-dopa-dyskinesias will be studied. This will be approached by (1) testing the hypothesis that a compromised nigrostriatal dopamine reuptake system predisposes to dopa-dyskinesias. This will be studied by initiating a drug-induced impairment of dopamine reuptake in normal animals to determine if there is an enhanced susceptibility to dopa-dyskinesias. (2) We will also investigate the relative roles of dopamine receptor subtypes (D1, D2 and D3) in the genesis of dopa-dyskinesias by administration of receptor subtype specific agonists and antagonists. (3) Our third specific aim will involve experiments to determine the integrity of the nigrostriatal system in the different groups of monkeys with dopa-dyskinesias. (4) Lastly, we will study the molecular events in the basal ganglia which mediate the development of dopa-dyskinesias using different models of dyskinesias described above. This will include alterations in dopamine receptor-linked coupling mechanism (such as dopamine-stimulated 35SGTPgammaS binding, DARPP-32 phosphorylation and adenylate cyclase activity), changes in NMDA receptor number and phosphorylation, and alterations in PPE mRNA levels. The results of this work will advance our understanding of the molecular mechanism responsible for the debilitating dyskinetic movements which occur as a consequence of long-term L-dopa treatment in Parkinson's disease. -

Principal Investigator: LANSBURY, PETER T
Grant Number: 1R21NS047420-01A1
Title: High Throughout Assay to Probe UCH-L1 Ligase Inhibitors

Abstract: Parkinson's disease (PD) is characterized by the presence of Lewy bodies (the cytoplasmic neuronal inclusions) and the significant loss of dopaminergic neurons in the substantia nigra, a-synuclein was identified as one major fibril component of the Lewy bodies, thus linked the accumulation of this protein to the pathogenesis of PD. Failure to regulate the concentration of a-synuclein, for example by dysfunction of the pathogenesis of PD. Failure to regulate the concentration of a-synuclein, for example by dysfunction of degradation process, can also contribute to the build-up and consequently fibrillation of the protein. A gene, PARK5, has been linked to PD are involved in proteasomal degradation pathway and it is an ubiquitin C terminal hydrolase (UCH-L 1) that hydrolyzes C-terminal ester and amides of ubiquitin and is believed to play a key role in processing polyubiquitin and/or ubiquity lated proteolytic peptide. A rare mutation (193M) of UCH L 1 that yields a 50% reduction in its hydrolytic activity has been tentatively linked to a rare early onset form of PD, at the same time a polymorphism of the enzyme (S 18Y) was indicated to reduce the risk of PD. The assumption that each enzyme expresses a single enzymatic activity in vivo, however, is challenged by the linkage of UCH-L 1 to PD. UCH-L 1, especially those variants linked to higher susceptibility to PD, causes the accumulation of a-synuclein in cultured cells, an effect that cannot be explained by its recognized hydrolase activity. UCH-L1 exhibits a second, dimerization-dependent, ubiquityl ligase activity. The polymorphic variant of UCH-L1 that is associated with decreased PD risk (S 18Y) has reduced ligase activity, but comparable hydrolase activity as the wild-type enzyme. Thus the ligase activity, as welt as the hydrolase activity of UCH-L1 may play a role in proteasomal protein degradation, a critical process for molecules ("molecular probes") that can be used to perturb UCH-L1 ligase activity in cell culture and animal models of PD. This "chemical genetic" strategy is complementary to traditional genetic approaches (e.g., knockouts and trasngenics) for understanding protein function but has a distinct advantage in that the probes are potential lead compounds for the development of novel PD therapetutics. The program detailed below will seek probes with the following activities: (1) inhibitors ofUCH-L1 dimerization, (2) inhibitors of UCH-L1 ligase activity, and (3) repressors and activators of UCH-L1 expression. -

Principal Investigator: LAU, YUEN-SUM
Grant Number: 5R01NS047920-02
Title: Impact of Exercise on Parkinson's Disease Therapy

Abstract: Parkinson's disease (PD) is a slow, progressive, debilitative, neurodegenerative disease, which has no cure. The current pharmacological therapies only temporarily mask symptoms, but do not protect neurons from further degeneration. Furthermore, chemotherapeutic agents often cause severe adverse effects and reduce the effectiveness of treatment. Numerous clinical reports have suggested that endurance exercise can slow down disease progression, and add years of independent and quality life to PD patients, or even improve the delivery and efficacy of L-DOPA treatment. Exercise therapy, or in conjunction with drug therapy at early onset of disease state, have been highly advocated by recent clinical trials. The potential health benefit and neurological mechanisms of action for exercise on PD rehabilitation have not been rigorously tested in the laboratory animal models. This research is designed to elucidate the impact of endurance exercise training on nigrostriatal dopamine (DA) neuron plasticity using a slow, progressive, and neurodegenerative mouse model of PD developed and characterized by our laboratory. This model is established based on a regimen of chronic 1-Methyl-4-phenyl - 1,2,3,6-tetrahydropyridine (MPTP) injections co-administered with probenecid, a drug that inhibits the peripheral and neuronal clearance of MPTP and potentiates the neurotoxicity of MPTP. In this model, we observed a marked decrease of nigrostriatal DA function within one week after treatment and remained low for 6 months. The animal also shows a gradual loss of substantia nigra (SN) neurons, decline of motor activity, and an accumulation of c-synuclein-immunoreactive inclusions in the SN. We further present in the application our preliminary findings supporting the feasibility and potential neuromodulatory role of endurance exercise on enhancing nigrostriatal DA transmission and PD rehabilitation using this model. In this research, we will test the following hypotheses centered on the endurance exercise, when administered at an early stage in the parkinsonian (PK) mice, will 1) improve their mobility and physical rehabilitation, 2) improve the efficacy of L-DOPA, 3) produce these effects by mechanistically causing an elevation of BDNF expression, an increase in the differentiation of DA progenitor cells, and an enhanced DA transmission and plasticity in the nigrostriatal neurons. Findings from this research should provide new insight into the development of alternative therapeutic approaches for enhancing the conventional pharmacological treatment and rehabilitation of PD. Potential benefits for using such a synergistic approach in managing PD would likely reduce the risk of drug toxicity and lower the cost of health

Principal Investigator: LAWRENCE, MATTHEW S
Grant Number: 1R43NS048786-01
Title: Genomic markers of environmental toxins for Parkinsonism

Abstract: Parkinson's disease is a prevalent and devastating neurodegenerative condition of unknown etiology. One prominent hypothesis holds that the selective loss of the nigrostriatal dopaminergic neurons characteristic of the disease results from damage from environmental neurotoxins in genetically vulnerable individuals. Identifying such environmental contributors to Parkinson s pathogenesis represents a significant public health concern. This project aims to identify the in vivo gene expression changes that occur in the primate brain in response to environmental toxins that have been implicated in the production of Parkinson's and compare these changes with the selective neurotoxin, MPTP, and with the limited knowledge of genetic abnormalities in some Parkinson's patients. Because of the unique vulnerabilities of nonhuman primates and humans to dopamine neurotoxic agents, studies in primates are essential to uncover common genetic markers of toxicity and to reveal the potential toxicity of chemicals of unknown liability. The proposed Phase I studies will test the hypotheses that transcriptional changes that accompany and precede dopamine cell death can be identified using high density gene arrays and bioinformatics in the primate nigrostriatal system in vivo following MPTP exposure. Changes in mRNA initiation of regimen of 3 doses of MPTP over 36 hours that has been established to result in Parkinsonism. Expression changes will also be assessed 6 hours after the administration of a single dose. Changes in nigrostriatal dopamine concentrations and tyrosine hydroxylase immunohistochemistry will be assessed at all time points. Additionally neurobehavioral changes will be assessed in the 20-day animals. Together these data will allow a determination of the sequence of transcriptional changes that parallel or precede histological, biochemical and behavioral events, and allow an assessment of transcriptional events related to acute versus chronic toxicity, with confirmation by quantitative RT-PCR. Defining the chronological and dose dependent gene expression changes induced by MPTP may reveal a transcriptional profile that is predictive of nigrostriatal injury from this toxin. Phase II studies will address whether similar gene expression changes and neuronal injury are seen following exposure to environmentally prevalent compounds that are postulated to be risk factors for the development of Parkinson's disease, and to integrate the resulting transcriptional data into a toxicogenornic database and potentially customized microarrays which may be applied to the assessment of compounds for their possible health risk.-

Principal Investigator: LEONARD,
Grant Number: 5R01NS027881-10
Title: Synaptic Modulation of Mesopontine Cholinergic Neurons

Abstract: Chronic or intermittent sleep disorders such as narcolepsy, sleep apnea, and insomnia afflict nearly 40 million people in the United States. Yet the neural mechanisms controlling both normal sleep and its pathologies remain poorly understood. Considerable evidence indicates that mesopontine cholinergic neurons are critical for this control and that their disregulation is involved in narcolepsy, Parkinson's disease, supranuclear palsy and depression. The long-term goal of this project is to understand the synaptic and non-synaptic mechanisms regulating activity of mesopontine cholinergic neurons. Recent compelling evidence indicates that disruption of the novel Hypocretin/Orexin (Hcrt/Orx) peptide system results in narcolepsy - a sleep disorder characterized by excessive daytime sleepiness, sleep fragmentation and the intrusion of rapid eye movement sleep behaviors into wakefulness. Anatomical evidence and our data indicate that mesopontine cholinergic neurons are important targets of these peptides. This proposal focuses on identifying the mechanisms by which Hcrt/Orx acts upon mesopontine cholinergic neurons and associated sleep-related neurons. We will investigate the general hypothesis that Hcrt/Orx peptides regulate both the short-term and long-term excitability of sleep-related neurons. To do so we will use whole-cell recording and calcium imaging methods in brain slices obtained from control mice and mice lacking the two known orexin receptors. We will address this hypothesis by 1) characterizing the ionic currents responsible for the post-synaptic excitatory actions of Hcrt/Orx peptides; 2) Identifying the sources and consequences of intracellular [Ca2+] changes produced by Hcrt/Orx peptides; 3) Identifying the specific roles of each orexin receptor by utilizing single and double receptor knockout mice and 4) Investigating possible alterations in neuron excitability in the mouse double orexin receptor knockout model of narcolepsy. Collectively, these results will advance the understanding of the molecular and cellular mechanisms underlying sleep regulation and its pathology. -

Principal Investigator: Levine, Michael S
Grant Number: 2R01NS033538-09
Title: Physiological Modulation by Dopamine in the Neostriatum

Abstract: The experiments in this proposal are designed to continue our investigations into cellular electrophysiological processes controlling dopamine (DA) modulation of responses mediated by activation of ionotropic glutamate receptors (iGluRs) in medium-sized spiny neurons of the striatum (MSSNs). The complex interactions between DA and iGluR-mediated neurotransmission within the striatum form the underpinnings of movement sequencing, motivation and reward responses, and psychological normalcy, just to provide a few examples. Imbalances in the interplay of these neurotransmitters have devastating consequences that are apparent in prevalent neurological and neuropsychiatric diseases such as Parkinson's and Huntington's diseases, attention deficit hyperactivity disorder (ADHD), schizophrenia, Tourette's syndrome, and many addictions. We have shown that DA, via D1 receptor activation enhances responses mediated by NMDA receptors while D2 receptor activation attenuates responses mediated by non-NMDA receptors (AMPA/KA). For example, when a D1 agonist was applied and a response was mediated by NMDA receptors, 98% of the time the response was enhanced. When a D2 agonist was applied and a response was mediated by non-NMDA receptors the response was attenuated 100% of the time. Other combinations (D2-DMDA, D1-non-NMDA) were less predictable. We will continue to focus on these interactions as an underlying theme, but will evaluate new areas pertaining to DA modulation. First, we will assess DA-iGluR interactions in a novel mouse model of ADHD that has the DA transporter (DAT) knocked down to 10% of basal levels. This produces a hyperDA state. Our working hypothesis is that DA modulation of iGluR transmission is altered in this genetic model and we have preliminary data to support it. Second, we will further examine mechanisms that control the predictability of DA modulation of GluR responses determining why the D2-NMDA and D1-non-NMDA receptor interactions are less predictable. Our hypothesis is that if factors controlling these interactions can be reduced, the interactions become predictable. We will use a novel mouse model in which enhanced green fluorescent protein is expressed under the control of the promoters for the D1 or D2 DA receptors or the M4 muscarinic acetylcholine receptor. This will allow electrophysiological recording in identified MSSNs that make up the direct or indirect output pathways of the striatum. Third, we will begin to dissect the NMDA receptor in MSSNs to determine how DA modulation is affected when selective subunits or their components (NR2A, NR2A-C-terminal, NR2B) have been removed or blocked pharmacologically. Our working hypothesis is

Principal Investigator: LI, LIAN
Grant Number: 1R01NS047199-01
Title: Characterization of a neuronal ubiquitination machinery

Abstract: Protein ubiquitination has emerged as a crucial mechanism for controlling development and function of neuronal circuits, and its defective regulation has been implicated in the pathogenesis of a variety of neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis. However, very little is presently known about the molecular machinery that controls protein ubiquitination in neurons. In the ubiquitin-proteasome pathway, substrate proteins are marked for degradation in the proteasome by covalent linkage to ubiquitin, a 76-amino acid polypeptide. The ubiquitination process involves a highly specific enzyme cascade in which ubiquitin is first activated by an E1 ubiquitin-activating enzyme, then transferred to an E2 ubiquitin-conjugating enzyme, and finally ligated to the substrate by an E3 ubiquitin-protein ligase. Of these enzymes, E3 ligase is the most important player because it determines the specificity of ubiquitin-mediated protein degradation. The importance of E3 ligases in neurodegenerative disorders is highlighted by recent findings that mutations in the E3 ligase parkin are responsible for a familial form of Parkinson's disease. In a search for neuronal proteins that regulate the neurotransmitter release machinery component SNAP-25, the applicant has discovered a novel protein, called Spring. Spring is a neuron-specific member of the RING-B-box-coiled-coil (RBCC) protein family. The importance of the RBCC family is underscored by the identification of the mutations in several RBCC proteins as the causes for a number of human diseases, including Opitz syndrome, Mulibrey nanism, and familial Mediterranean fever. In this project, the applicant will use a combination of biochemical, proteomic, molecular biological, and cell biological approaches to test the hypothesis that Spring functions as a novel E3 ubiquitin-protein ligase to regulate the turnover of the neurotransmitter release machinery. In addition, this project will characterize neuronal distribution and synaptic localization of Spring, and explore the possible involvement of this novel protein in Alzheimer's disease and Parkinson's disease. Successful completion of proposed studies will yield novel insights into the molecular mechanisms that control neuronal protein ubiquitination and neurotransmitter release, and provide fundamental information towards our ultimate goal of understanding and treating numerous neurological diseases and psychiatric disorders.-

Principal Investigator: LINDSEY, BRUCE G
Grant Number: 2R37NS019814-19A1
Title: Brainstem Respiratory Neuron Interactions

Abstract: Understanding the brainstem network that generates and modulates the respiratory motor pattern is an important goal in physiology and medicine. Disorders that disrupt breathing are associated with the development of pulmonary and systemic hypertension, sudden infant death, learning disabilities, Parkinson's disease and autonomic dysfunction, and other disorders and risks, e.g., stroke and multiple sclerosis. A neural network in the ventrolateral medulla is considered essential for generating the respiratory motor pattern. The circuits used by other brainstem sites to modulate this network are not known. The primary hypothesis of this project is: Peripheral and central chemoreceptors modulate the respiratory motor pattern through a distributed network that includes multifunctional neurons in the "pontine respiratory group" (PRG), the medullary raphe nuclei, and the locus ceruleus. The project has four specific aims: 1. Define functional circuits within the pontine respiratory group (PRG) and between the PRG and the core medullary respiratory network. 2. Define the functional circuits of the PRG and the core medullary respiratory network appropriate for modulation of the respiratory motor pattern during changes in central and peripheral chemoreceptor drive. 3. Define the functional connectivity of raphe neurons that respond to changes in central and peripheral chemoreceptor drive, including functional circuits between the raphe system and the PRG and core medullary networks. 4. Identify functional circuits of locus ceruleus neurons that respond to changes in central and peripheral chemoreceptor drive and their functional connectivity with other modulatory domains and the core medullary respiratory network. The project will use multi-array recordings and computational methods to define functional circuits and their responses during chemoreceptor challenges that alter breathing. The results will lead to new network models of the respiratory brainstem in both normal and pathophysiological conditions.-

Principal Investigator: MARSHALL, JOHN F
Grant Number: 5R01NS022698-15
Title: Striatal organization and dopamine receptor localization

Abstract: The globus pallidus (GP; external pallidum of primates) plays a key role in the circuitry of the basal ganglia. It receives synaptic input from all of the striatal spiny projection neurons and distributes axon collaterals to all structures of this circuit, including the striatum. Recent research highlights the diversity of its neurons, with many containing parvalbumin (PV) and others expressing preproenkephalin (PPE) mRNA. The dopaminergic innervation of the pallidum is functional, because intrapallidal infusions of dopamine (DA) or DA antagonists alter unit firing and affect GP immediate early gene expression. The influences of D2-class DA antagonists on immediate early gene expression occur predominantly within the PPE+/-/(PV-) pallidostriatal cells. The mRNA for the D2 dopamine receptor is expressed by many (48 percent) of the neurons in rodent GP, making this nucleus a likely target for actions of exogenously administered dopaminergic agents (e.g., L-dopa in Parkinson's disease). Four specific aims will address the issues of neuronal heterogeneity in GP neuron populations and the actions of DA on pallidal neuron function. These aims include: (1) studying pallidal immediate early gene response to local infusions of D2-, D3-, and D4-preferring DA antagonists and characterizing D3 mRNA-expressing neurons according to their axonal projections, their PPE mRNA, and their immediate early gene response; (2) investigating interactions between subthalamic nucleus activation or inactivation and local pallidal effects of D2-class agonists or antagonists, (3) determining the expression of GAD67 mRNA within identified populations of GP neurons after DA cell injury or DA antagonist treatment to determine cell type-specific regulation of this transmitter-related late gene; and (4) testing the hypothesis that the pallidostriatal axon collaterals influence gene expression within striatal PV interneurons. This last aim uses a paradigm of cortically-driven c-fos induction in striatal PV and enkephalin neurons to investigate whether intrapallidal GABA-A or D2 receptor drug infusions can suppress or enhance c-fos induction within these striatal interneuron and projection neuron populations. These experiments should substantially advance our understanding of (1) the phenotypic diversity of GP neurons, and (2) the actions of DA in the globus pallidus. The proposed research focuses on the GP neurons that contain D2 or D3 mRNA and/or are altered by the administration of DA antagonists. The significance of this work derives both from its relevance to basal ganglia disorders such as Parkinson's disease and also because of its potential to elucidate the significance of the pallidostriatal circuitry. -

Principal Investigator: MCDONALD, PAUL W
Grant Number: 5F31NS046237-02
Title: Using C. elegans to Investigate the Dopamine Transporter

Abstract: The dopamine (DA) transporter (DAT) is critical in the re-uptake of DA into presynaptic neurons and the termination of dopamine signaling. Alterations in DA signaling is evident in several disease pathologies including ADHD, Parkinson's disease and schizophrenia. Recently, it has been shown that intracellular proteins interact with and affect the localization and function of DAT. As such, we hypothesize that DAT exists in a protein complex that regulates the activity of the transporter. To test this hypothesis we will take advantage of the model system C. elegans to test candidate regulatory proteins for DAT and identify novel proteins that associate with the transporter. The C. elegans dopamine transporter DAT-1) shows a 45% homology to the human transporter, with a sensitivity to amphetamines, cocaine, and other biogenic amine transporter antagonists. In my proposal, I build on recent studies by our laboratory on DAT-1 to: 1) determine the expression level and localization of DAT-1, 2) characterize the interactions of the C. elegans PICK1 homologue (Y57G11C.22) with DAT-1 and, 3) identify novel proteins that interact with the C. elegans dopamine transporter. These studies will increase our understanding of intrinsic modulatory influences controlling DA signaling in vivo and in disease states.-

Principal Investigator: Mckay, Ronald
Grant Number: 5Z01NS002981-06
Title: Stem Cell Biology And Brain Disease

Abstract: Unavailable

Principal Investigator: MCNAUGHT, KEVIN S
Grant Number: 1R01NS045999-01A1
Title: ROLE OF PROTEASOMAL DYSFUNCTION IN PARKINSON'S DISEASE

Abstract: Parkinson's disease is characterized pathologically by selective degeneration of dopamine-containing neurons in the substantia nigra pars compacta (SNc). The etiology in the vast majority of individuals with the disorder remains elusive but ageing is an important risk factor. Nigral cell death in PD is accompanied by the accumulation of oxidatively damaged proteins, aggregation of proteins and the formation of proteinaceous intracytoplasmic Lewy body inclusions. These observations suggest that failure of the ubiquitin-proteasome system (UPS), the biochemical pathway primarily responsible for the degradation of abnormal and short-lived regulatory/transcriptional proteins may underlie nigral pathology in Parkinson's disease. Indeed, mutations in the genes encoding alpha-synuclein and 2 enzymes of the UPS, namely parkin and ubiquitin Cterminal hydrolase L1, are associated with altered protein handling in rare familial forms of Parkinson's disease. However, these or similar gene defects do not occur in most patients who have sporadic Parkinson's disease. We hypothesize that defects in 26/20S proteasomes cause the UPS to fail and this underlies protein accumulation, Lewy body formation and dopaminergic neuronal death in the SNc in sporadic Parkinson's disease. Consistent with this hypothesis, our preliminary findings demonstrated structural and function defects in 26/20S proteasomes, and a several-fold increase in the levels of poorly degraded/undegraded and potentially cytotoxic ubiquitinated protein substrates, in the SNc but not elsewhere in sporadic Parkinson's disease. In addition, we showed that in aged control subjects and adult rats, dopaminergic neurons of the SNc have relatively low 26/20S proteasomal activity and poor expression of the proteasome activators (PA28 and PA700) compared to other brain regions. Further, we showed that inhibition of 26/20S proteasomal function causes selective degeneration of dopaminergic neurons with the formation of alpha-synuclein/ubiquitin-immunoreactive inclusions in primary mesencephalic cultures and in rat SNc with motor dysfunction. In this project, we propose to determine (1) if and how the structure and function of proteasomes are defective in all stages of sporadic PD; (2) if low proteasomal function normally occurs in the SNc of controls as this may underlie its selective vulnerability and degeneration in PD; (3) if proteasomal dysfunction underlies Lewy body formation; and (4) if proteasomal dysfunction plays a role in nigral dopaminergic cell death in sporadic Parkinson's disease. These studies will test our hypothesis that inadequate proteasomal function underlies both vulnerability and degeneration of the SNc in sporadic Parkinson's disease.-

Principal Investigator: Meredith, Gloria
Grant Number: 5R01NS041799-05
Title: Synaptic Proteins, Trophic Factors and Neurodegeneration

Abstract: One of the most fundamental questions related to the progressive nature of neurodegeneration in human disease is how neurons die. Protecting nerve cells against morphological decline and death requires blocking intrinsic factors that inhibit neural repair. In the present proposal, we offer an innovative approach to study those factors that are active in Parkinson's disease (PD) in a new mouse model that shows synaptic loss and irreversible nigrostriatal degeneration. We propose to track changes of a key synaptic protein, a-synuclein, both in its native environment at presynaptic terminals and under neurotoxic conditions, when it becomes insoluble and accumulates. We will further correlate those changes with altered neurotrophic support. We have established an animal protocol by treating C57/bl mice with a combined regimen of 10 doses of probenecid at 250mg/kg and MPTP at 25mg/kg for 5 weeks. These mice show a slow, progressive loss of nigrostriatal dopaminergic function for at least 6 months, that mimics PD, with no signs of recovery. Three weeks after drug treatment, there is a significant reduction in the number of substantia nigra (SN) cells and dramatic changes in the subsynaptic distribution and density of a-synuclein-immunoreactive terminals. These changes could signal the beginning of a chain of events that leads to cell death. In this proposal, we will focus on the progressive deterioration of dopaminergic neurons in the SN and their inputs, and present three specific aims to be addressed through a series of hypotheses. Specifically, we plan to 1) ascertain the origin and neurochemical phenotype of synapses in the SN that contain a-synuclein and to establish whether MPTP + probenecid treatment leads to their degeneration; 2) determine, in the MPTP+P model, the temporal relationships between cell death and a-synuclein-positive synapses, decline in dopamine function and behavior; and 3) ascertain whether changes in a-synuclein expression and production are precipitated by altered neurotrophic support. The overall objective of our research is to understand the relationship between the synaptic protein, a-synuclein, neurotrophic support, especially brain-derived neurotrophic factor (BDNF) and their respective roles in the PD form of neurodegeneration. The findings of this research should shed light on target areas where neuroprotection strategies can be implemented. -

Principal Investigator: MOSLEY, RODNEY L
Grant Number: 1R21NS049264-01
Title: Neuroprotective Vaccination for Parkinson's Disease

Abstract: Microglia inflammation contributes, in significant measure, to the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) during idiopathic Parkinson's disease (PD). Attenuation of such inflammation could attenuate disease. To this end we show that microglial deactivation responses, induced by vaccination, in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) intoxicated mice improves dopaminergic neuronal survival. This was achieved by adoptively transferring spleen cells from copolymer-1 (Cop-1) immunized mice to MPTP-treated recipients. Spleen cells from ovalbumin (OVA) injected mice failed to affect neuronal protection. Thus, our preliminary works show that protection from dopaminergic neurodegeneration can be achieved by adaptive immunity with T cells specific for Cop-1. Based on response kinetics, antigen specificity, and functional adaptive T cell immune responses, we predict that the mechanism(s) of neuroprotective immunity can be realized and could provide novel treatment strategies for human disease. Our hypothesis posits that protection from dopaminergic neurodegeneration by Cop-1 vaccination is generated through immune cell-mediated mechanisms with specificity for Cop-1 peptides and self-antigens. To investigate this we will adoptively transfer T lymphocytes, B cells and monocytes from Cop-1 immunized mice into MPTP-treated animals. Neuroprotection will be assessed by numbers of dopaminergic neurons, neurotransmitter levels, and neuronal metabolites by magnetic resonance spectroscopic imaging (MRSI). Immune cell populations, proven relevant to neuroprotection will be evaluated for the expression of gene products that are cell population specific as candidates for neuroprotection. Genetic fingerprint analysis will include cDNA microarray analysis and proteomics. This approach takes advantage of an integrated and well-established research program within the Center for Neurovirology and Neurodegenerative Disorders and builds upon research activities in PD supported previously through private donations. These approaches could prove useful for treatment of human PD. -

Principal Investigator: MUCHOWSKI, PAUL J
Grant Number: 1R01NS047237-01
Title: Modifiers of Huntingtin and Alpha-synuclein Toxicity

Abstract: Huntington's disease (HD) is an autosomal dominant inherited disorder characterized by involuntary movements, personality changes and dementia, and is caused by an expansion of a CAG/polyglutamine repeat in the IT-15 gene. A major neuropathological hallmark in HD is the occurrence of intranuclear and cytoplasmic inclusion bodies that contain huntingtin (the protein encoded by IT-15). Cytoplasmic inclusion bodies (Lewy bodies) are also a prominent nature of Parkinson's disease (PD), a neurodegenerative disorder characterized by muscle rigidity, bradykinesia, resting tremor and postural instability. Lewy bodies are composed primarily of the protein alpha-synuclein, and two point mutations in the alpha-synuclein gene cause early-onset, inherited forms of Parkinson's disease. Alpha-synuclein and huntingtin aggregate into ordered fibrillar structures with properties characteristic of amyloid. The 'amyloid hypothesis', developed originally to describe the role of beta-amyloid in Alzheimer's Disease (AD), suggests that the aggregation of proteins into an ordered fibrillar structure is causally related to aberrant protein interactions that culminate in neuronal dysfunction and cell death (Hardy and Selkoe, 2002). The precise roles of protein aggregation, amyloid formation and inclusion bodies in neurodegeneration remain controversial, and it is not yet clear if common molecular mechanisms underlie HD and Parkinson's disease. We have used yeast as a model eukaryotic organism to test the hypothesis that the downstream targets and molecular mechanisms by which huntingtin and ot-synuclein mediate toxicity are unique. Using a genome-wide screening approach in yeast we isolated 52 genes that modify huntingtin toxicity, and 86 genes that modify alpha-synuclein toxicity. 30% of genes that affect huntingtin toxicity are enriched in the functionally related categories of protein folding and cell stress, while 29% of genes that modify alpha-synuclein toxicity are involved in vesicular transport and lipid metabolism. Our preliminary results indicate surprisingly that the genes and cellular pathways that modulate huntingtin and alpha-synuclein toxicity in yeast are completely divergent. Nearly half of the genes we isolated are annotated as having one or more human ortholog, suggesting we may have discovered in yeast conserved cell-biological response pathways to huntingtin and alpha-synuclein that are relevant to HD and Parkinson's disease. Using the resources and information that we have generated, we now wish to advance our understanding of the neurodegeneration that occurs in HD and PD by applying molecular genetic and biochemical techniques to validate (or invalidate) the genetic modifiers we have identified. Our

Principal Investigator: PAPA, STELLA M
Grant Number: 1R01NS045962-01A1
Title: Regulation of Motor Function in Parkinson's Disease

Abstract: Motor disturbances of Parkinson's disease are caused by a series of functional alterations in the basal ganglia that derive from dopamine denervation. The mechanisms underlying those functional alterations are not completely understood yet. Moreover, long-term levodopa therapy is usually associated with disabling motor complications, such as motor fluctuations and dyskinesias, whose pathophysiology also remains obscure. The long-term objective of this project is to elucidate the pathophysiologic mechanisms of abnormal motor behaviors in Parkinson's disease in view of developing new and specific therapeutic tools. Thus, this study is aimed: -firstly, to localize functional alterations in specific basal ganglia circuits; -secondly, to determine the glutamate regulation associated to an altered neuronal function; -finally, and based on the foregoing data, to explore new therapeutic approaches by interacting with the glutamatergic neurotransmission in a region-specific manner. Specifically this project comprises three aims: 1. To study the neuronal activity of individual basal ganglia regions by single cell recording in normal and various groups of parkinsonian monkeys (MPTP-treated primates) that exhibit different motor behaviors depending on treatment conditions (i.e.: parkinsonian state, its normalization, and drug-induced dyskinesias). 2. To study the glutamate receptor sensitivity in basal ganglia regions in relation to different motor conditions by comparing the binding of receptors across animal groups. 3. To study the glutamatergic blockade in restricted basal ganglia regions by determining its effects on neuronal activity and motor behavior. The research design includes techniques ranging from single- and multiple single- unit recording of neuronal activity, autoradiographic binding of receptors, to intracerebral administration of drugs in parkinsonian monkeys whose motor abnormalities closely resemble the human disease. This project proposes a novel approach to a comprehensive study of the abnormal motor function in Parkinson's disease. Thus, it will largely contribute to the rationale for new treatments that selectively target particular motor conditions. -

Principal Investigator: PARNG, CHUENLEI
Grant Number: 1R43NS048607-01
Title: In Vivo Screen for Neuroprotective Agents

Abstract: Aberrant apoptosis is implicated in several neurodegenerative disorders including, stroke, brain trauma, spinal cord injury, Parkinson's disease, amyotrophic lateral sclerosis (ALS), Alzheimer's and Huntington's disease. These neurodegenerative diseases are associated with high morbidity and mortality, and treatment options are limited. Agents that modulate apoptosis are a major focus of drug development efforts by biopharmaceutical companies. Assessment of drug effects in a convenient vertebrate model, prior to proceeding to evaluation in complex systems, such as mouse, can potentially streamline drug development and dramatically reduce costs. Zebrafish mutants exhibiting aberrant apoptosis in the central nervous system are an excellent animal model for studying neurodegeneration. Using a zebrafish neurodegenerative mutant line and a vital dye apoptosis assay, this Small Business Innovation Research project proposes to characterize embryogenesis and apoptotic patterning in zebrafish embryos, and to develop a rapid and effective in vivo screen for neuroprotective therapeutics.-

Principal Investigator: PATEL, MANISHA
Grant Number: 1R01NS045748-01A1
Title: Mitochondrial Aconitase and Parkinson's Disease

Abstract: The long-term goal of this proposal is to elucidate the mechanism by which mitochondrial oxidative stress produces dopaminergic neuronal death in Parkinson's Disease (PD). The precise mechanism by which mitochondrial oxidative stress, bioenergetic decline and iron overload arise and collaborate to produce age-related neuronal death in Parkinson's disease remains unclear. It is hypothesized that neuronal damage in Parkinson's disease results, in part from direct superoxide radical toxicity due to oxidative inactivation of mitochondrial aconitase. The hypothesis predicts that superoxide production, arising from Complex I inhibition or abnormal dopamine metabolism, inactivates [4Fe-4S]Z+-containing mitochondrial aconitase, resulting in loss of aconitase activity and release Fe z+ and H202. Posttranslational modification of this key TCA cycle enzyme can therefore result in an increased iron load, oxidant burden and bioenergetic decline. The presence of an iron responsive element (IRE) in the 5' untranslated region of the mitochondrial aconitase Mrna provides an additional mechanism for iron dysregulation in Parkinson's disease. The proposal will utilize human PD samples, animal models of PD (1-methyl-4-phenyl-l,2,3,6-tetrahydropyridine and 6-hydroxydopamine) and dopaminergic cell culture models in conjunction with a diversity of tools and techniques that include biochemical analyses, confocal microscopy, molecular biology and transgenic/knockout/aging mice. Specific Aim 1 will determine whether mitochondrial aconitase is inactivated in human and experimental Parkinson's disease. The influence of aging and chronic mitochondrial oxidative stress will be determined using mice deficient in MnSOD, a critical mitochondrial antioxidant. Specific Aim 2 will determine whether mitochondrial aconitase inactivation contributes to impaired iron homeostasis. Specific Aim 3 will determine whether scavenging mitochondrial superoxide using native or synthetic antioxidants (e.g. MnSOD transgenic mice or metalloporphyrins) protect against mitochondrial aconitase inactivation in a manner that correlates with decreased iron overload and neuronal death in experimental Parkinson's disease. Specific Aim 4 will determine the downstream consequences of mitochondrial aconitase inactivation in experimental Parkinson's disease. Specifically, regulation of brain mitochondrial aconitase synthesis by the 5' IRE in its mRNA, impact on the TCA cycle capacity and direct neurotoxicity of aconitase gene silencing will be examined. These studies can advance our understanding of the oxidative mechanisms of neuronal death in Parkinson's disease and suggest novel therapeutic strategies

Principal Investigator: PERLMUTTER, JOEL S
Grant Number: 2R01NS041509-04
Title: MECHANISM OF DEEP BRAIN STIMULATION

Abstract: Deep brain stimulation (DBS) of the subthalamic nuclei (STN) may provide substantial reduction of symptoms in people with Parkinson disease (PD) and DBS of the thalamic ventral intermediate nucleus (VIM) markedly reduces tremor in people with disorders such as essential tremor (ET). Increasing data also indicates that STN DBS in PD may produce unwanted cognitive impairments, such as impairments of spatial delayed recall or response inhibition. Despite these dramatic clinical effects, the precise mechanism of action of DBS remains unclear. Recent studies, including several from this lab, indicate that DBS produces a net increase in neuronal output from the site of stimulation either the STN in PD or VIM in ET, and there may be important differences in the effects of STN on the left and right sides of the brain. Nevertheless, how this action and its asymmetry provide clinical benefit while simultaneously interfering with selected cognitive function remains unknown. We hypothesize that STN and VIM DBS provide motor benefit by altering function of specific motor brain regions, whereas, STN DBS impairs cognitive skills by altering function of selected prefrontal regions. Further, we propose that there are substantial differences between left and right-sided STN stimulation on aspects of motor and cognitive function. We will test these specific hypotheses using PET to measure brain blood flow responses to varying levels of STN or VIM stimulation in people with PD or ET and then correlate these PET responses with cognitive or motor responses to DBS in the same subjects. These studies have the potential to reveal valuable insights into the mechanism of DBS and also into the pathophysiology of these diseases and their clinical manifestations. For example, we may identify specific brain pathways that mediate cognitive impairment from STN DBS that are distinct from those that mediate motor benefit. This could directly lead to designing new strategies to maximize motor benefit and minimize cognitive impairments. We also have the potential to provide a rationale for investigating new sites for DBS that may be more accessible than those currently used. This innovative study brings together rigorous, carefully controlled PET investigations with quantified motor and cognitive behavioral measures.-

Principal Investigator: POISIK, OLGA
Grant Number: 5F31NS045458-02
Title: Metabotropic Receptors in Globus Pallidus

Abstract: Unavailable

Principal Investigator: QUIK, MARYKA
Grant Number: 5R01NS042091-02
Title: Nicotinic Receptors in Parkinson's Disease

Abstract: Our goal is to determine the role of nicotinic receptor subtypes in the basal ganglia with the long-term objective of developing novel therapeutic strategies for Parkinson's disease. The rationale for this work is based on studies showing that nicotine administration improves locomotor deficits after a nigrostriatal lesion and, furthermore, that nicotine or smoking results in an apparent protective effect against nigrostriatal damage in rodents and in Parkinson's disease. However, multiple nicotinic receptors are activated by nicotine to result in beneficial but also side effects in both the peripheral and central nervous system. We hypothesize that specific nicotinic receptor populations in the brain are involved based on work demonstrating that the distinct subtypes have unique localization and molecular functions. We will approach these studies through four specific aims. As a crucial first step, we will identify the regional and cellular localization of nicotinic receptor subtypes in basal ganglia and determine the changes in receptor subtypes after nigrostriatal degeneration. This will be done using three different approaches including in situ hybridization, receptor autoradiography and immunocytochemistry and form the basis of Specific Aims 1 and 2. We will then initiate studies to assess the nicotinic receptor subtypes involved in striatal function as described in Specific Aim 3, followed by behavioral studies (Specific Aim 4) to determine the ability of nicotinic agonists to reverse parkinsonism. This work should increase our understanding of the role of different nicotinic receptor subtypes in basal ganglia function. In summary, the proposed work should provide new insight concerning alterations in nicotinic receptor subtypes after nigrostriatal damage and nicotinic agonist treatment. These data together with the results of the behavioral studies may form a basis for the use of nicotinic drugs in the treatment of Parkinson's disease. -

Principal Investigator: RAGOZZINO, MICHAEL E
Grant Number: 5R01NS043283-02
Title: Striatal Acetylcholine and Behavioral Flexibility

Abstract: The main objective of this proposal is to build a greater understanding of how the striatal cholinergic system contributes to behavioral flexibility. There is accumulating evidence that neurological and psychiatric disorders that lead to striatal neuropathology, i.e. Parkinson's disease, Huntington's disease and schizophrenia, produce severe deficits in cognitive flexibility. In addition to the common cognitive symptomology, Parkinson's and Huntington's disease patients both exhibit decreases in cholinergic markers in the anterior regions of the caudate and putamen. At present, unknown is what striatal circuitry or neurochemical mechanisms underlie cognitive flexibility. Advances in elucidating the etiology of these disorders and development of effective treatments for the cognitive deficits relies, in part, on identifying the basic neurochemical mechanisms within the striatum that underlie the cognitive functions impaired in Parkinson's and Huntington's disease. The first goal of the proposal is to understand the dynamic changes in acetylcholine output in the dorsomedial and dorsolateral striatum during acquisition and reversal learning of a visual cue discrimination, using in vivo microdialysis with high pressure liquid chromatography. Recent timings in Parkinson's disease patients suggest that anti-cholinergic treatments lead to cognitive flexibility deficits. The second goal of the proposal is to determine whether specific muscarinic receptor subtypes in the dorsomedial striatum contribute to behavioral flexibility. Previous studies found that dopamine activity in the striatum also influences cognitive flexibility. Furthermore, extant research indicates an interaction between the dopaminergic and cholinergic systems in the basal ganglia related to motor behavior. The third goal of the proposal is to determine whether dopamine D1 and/or D2 receptors modulate acetylcholine efflux in the dorsomedial striatum to influence behavioral flexibility. Overall, this approach takes a unique approach in examining the dynamic changes in striatal acetylcholine release during the actual learning and shifting of strategies. The proposed studies will also provide complimentary information on the specific muscarinic receptors that may facilitate behavioral flexibility in the dorsomedial striatum. Moreover, the proposed studies can help unravel the complex interaction of neurotransmitters in specific striatal circuitry as it relates to behavioral flexibility. The findings from these experiments may enable the development of selective and targeted pharmacological interventions to alleviate the cognitive symptomology in Parkinson's and Huntington's disease without producing unwanted motoric side effects. -

Principal Investigator: RICE, MARGARET E
Grant Number: 5R01NS036362-07
Title: Electrochemical Analysis of Dendritic Dopamine Release

Abstract: Dopamine (DA) is a key transmitter in motor and emotive pathways of the brain. Dysfunction of dopaminergic neurotransmission underlies a variety of brain disorders that have a critical impact on society, including Parkinson's and Huntington's diseases, schizophrenia, and addiction. DA cell groups in the midbrain provide the primary source of DA to the CNS. Cells of the substantia nigra pars compacta (SNc) project rostrally to innervate the dorsal striatum (nigrostriatal DA system) whereas those of the adjacent ventral tegmental area (VTA) project to the nucleus accumbens and other limbic structures (mesolimbic DA system). A potentially unique characteristic of DA neurons is that they release DA from their dendrites in midbrain, as well as from distant axon terminals. Dendritic, as well as terminal release of DA is critical for DA-mediated behaviors, yet little is known about factors that regulate DA release In midbrain. Using carbon-fiber microelectrodes and fast-scan cyclic voltammetry to detect evoked DA release in real-time in brain slices, we have found significant differences in the Ca2+-dependence and Ca2+-channels required for dendritic vs. terminal release. Further, we have found distinct patterns of regulation by glutamate and GABA acting at ionotropic receptors in SNc, VTA and striatum. Significantly, we also discovered a novel, endogenous regulator of the nigrostriatal DA release: the reactive oxygen species (ROS), H202. In this continuation, we will investigate the distinct Ca2+ dependence of dendritic DA release, with emphasis on contributions from synaptic input to DA cells and release of Ca2+ from intracellular stores (Aim I). We will also elucidate the role of ROS as modulators of dendritic and terminal DA overflow (Aim ll-IV). Aim II will examine the involvement of H202 and other ROS in nigrostriatal vs. mesolimbic DA systems. Aim Ill will investigate sources of ROS, including terminals and cells adjacent to DA release sites. Aim IV will test whether H202 inhibits dendritic DA overflow by causing hyperpolarization of DA cells; effects on membrane properties will be indicated by whole cell recording. -

Principal Investigator: RICE, MARGARET E
Grant Number: 5R21NS045325-02
Title: Regulation of Dopamine Release by ROS

Abstract: Dopamine (DA) is a key modulator of motor and emotive pathways in the brain. Forebrain structures receive DA input exclusively from midbrain DA neurons, with cells of the substantia nigra pars compacta (SNc) projecting via the nigrostriatal pathway to dorsal striatum and those of the adjacent ventral tegmental area (VTA) project via the mesolimbic pathway to nucleus accumbens and other limbic structures. DA cells in both systems share common physiological properties, including somatodendritic release of DA. A significant difference, however, is that nigrostriatal DA cells degenerate in Parkinson's disease, whereas mesolimbic DA cells are spared. Several biochemical differences may contribute to greater SNc vulnerability, including weaker regulation of reactive oxygen species (ROS) in SNc than in VTA. This difference may be crucial, because oxidative stress has been proposed as a causal factor in Parkinson's disease. In addition to being potentially neurotoxic, however, ROS can act as signaling agents. Preliminary data implicate one ROS, hydrogen peroxide (H202), as a modulator of DA release. When applied exogenously, H202 inhibits release in the SNc and VTA as well as in dorsal striatum and nucleus accumbens. Additional data suggest that endogenous H202 generated during local stimulation inhibits DA release in the SNc and striatum, but not in the VTA. These data may reveal a normal physiological process in the nigrostriatal DA system that could contribute to oxidative stress, if regulation of H202 became disrupted. It is not clear, however, whether H202 per se is the modulator or whether it acts via related ROS. The goal of this R21 proposal is to determine specific ROS involved in modulating somatodendritic and synaptic DA release in the nigrostriatal vs. mesolimbic DA systems. Evoked DA release will be monitored using evoked using carbon-fiber microelectrodes and fast-scan cyclic voltammetry. Experiments in Aim t will compare regulation of somatodendritic DA release by ROS in the SNc and VTA, whereas those in Aim 2 will compare regulation in the dorsal striatum and the shell of the nucleus accumbens. Surprisingly, the studies proposed here would be among the first to investigate functional differences in ROS regulation between vulnerable and resistant DA systems. Consistent with the goals of the R21 program, these data should provide ground-breaking new information about underlying mechanisms in nigrostriatal degeneration. -

Principal Investigator: RODRIGUEZ, ALICE L
Grant Number: 1F32NS049865-01
Title: Development of allosteric potentiators of mGluR4

Abstract: Treatment of Parkinson's disease (PD) has traditionally focused on dopamine replacement strategies such as LDOPA. While generally effective early on, L-DOPA has often proven inadequate for long term treatment due to serious adverse side effects. Recent studies in Dr. Conn's laboratory suggest that activators of metabotropic glutamate receptor mGluR4 may provide a novel pharmacological approach to the treatment of PD by targeting the indirect pathway of the basal ganglia. Furthermore, Dr. Conn and coworkers have developed a novel approach to activation of mGluR4 by development of allosteric potentiators that do not activate this receptor directly but dramatically potentiate the response to glutamate. While these studies provide an exciting proof of principle for a novel approach to activation of mGluR4, there is a need to develop novel compounds that have a higher potency and are useful for further in vivo studies. The goal of this work is to develop novel potent and selective allosteric potentiators of mGluR4. A threefold approach will be implemented, beginning with performing a high throughput screen mining for compounds that potentiate the glutamate response of mGluR4. In parallel with the HTS, medicinal chemistry studies will be pursued to improve upon the properties of known potentiators. Finally, mutagenesis studies will be performed to develop a better understanding of the molecular interactions involved in potentiator binding which will subsequently aid in the design of future compounds. Together these approaches will result in the development of novel small molecules that have a therapeutic effect on PD by reducing transmission through the indirect pathway. Furthermore, these studies will be complemented by ongoing electrophysiology and behavioral studies in Dr. Conn's laboratory that will determine the effects of these compounds in vitro models of basal ganglia function. -

Principal Investigator: RUOHO, ARNOLD E
Grant Number: 5R01NS033650-09
Title: Characterization of Vesicular Monoamine Transporters

Abstract: The strategy of this proposal is based on the rationale that identification of the inhibitor, substrate, proton translocation, and functionally relevant phosphorylation sites on monoamine transporters (VMAT2) will provide a basic understanding of the mechanism of action of monoamine sequestration into vesicles and the factors which regulate transporter activity. This work will be accomplished in three Specific Aims: (1) Identification of the reserpine binding site(s) on VMAT2. Novel reserpine photoaffinity labels will be synthesized and characterized, and photo-labelled peptides will be identified in order to map the reserpine binding site; (2) Identification of the substrate transport channel. This aim will involve the use of several approaches, including radioactive photo-activatable substrate analogs to covalently derivatize the substrate binding site on VMAT2; site-specific derivatization of VMAT2 at engineered cysteine residues with the cysteine-reactive reagents, methanethiosulfonate ethyl amine (MTSEA), and MTS-ethyltrimethylammonium (MTSET); and site-directed mutagenesis of potential residues lining the channel; (3) Determination of the functional role of two highly charged regions of VMAT2. This aim will involve the use of biochemical and genetic (site-directed mutagenesis) approaches to determine the role of phosphorylation of the N-terminus of VMAT2 on transporter function and the intracellular distribution/oligomeric state of the transporter. Reduced or aberrant activity of the monoamine transporter of the synaptic vesicles in dopaminergic neurons of the substantia nigra through either direct or indirect actions of toxicants (e.g., MPP+, insecticides) and genetically altered neuronally expressed proteins may play a central role in Parkinson's Disease. The regulation of uptake of monoamine neurotransmitters into storage vesicles may also play an important role in affective psychological disorders related to depression by altering levels of serotonin, norepinephrine, dopamine, or other neurotransmitters. This work will provide insight into the mechanism of action of the monoamine transporters and contribute to our understanding of how pharmacological and therapeutic strategies may be devised to treat Parkinsonism or other disorders of the nervous system. -

Principal Investigator: RYE, DAVID B
Grant Number: 5R01NS043374-03
Title: Circuitry of Midbrain Dopamine in Sleep & Wake

Abstract: Dopamine (DA) is a neurotransmitter that modulates diverse waking behaviors including movement, motivation, cognition, reward, and feeding. Less appreciated and understood are dopamine's influences upon normal and pathologic sleep. Dopamine cell death which occurs in Parkinson's disease, is associated with profound alterations in wake-sleep state that can be broadly classified into disturbances of noctural movement and thalamocortical rhythmicity. The former include periodic leg movements of sleep and rapid-eye-movement sleep (REM-sleep) behavior disorder, while the later encompass loss of sleep spindles and slow-wave sleep, daytime sleepiness, and daytime intrusion of REM-sleep manifesting as hallucinatory behavior. Heuristic models of disease have limited themselves largely to DA's indirect, rather than direct actions upon thalamocortical circuits, and also to DA's participation in waking behaviors rather than thalamocortical arousal state (e.g., sleep). We have recently described a novel mesothalamic dopamine pathway originating via axon collaterals of the nigrostriatal pathway and which degenerates in PD. Mesencephalic dopamine neurons therefore have potential to modulate normal and pathologic behavior, including sleep, not only through traditional nigrostriatal pathways, but also by way of axon collaterals to the thalamus. Here we propose to define anatomical and physiological features of these novel circuits in non-human primates. S.A. number 1 employs microscopic techniques to establish the distribution, subcellular targets, topography, and collateralization of DA innervation in "motor", "prefrontal", "limbic" and the reticular (i.e., the thalamic pacemaker) thalamic nuclei. S.A. number 2 will extend our preliminary electrophysiological demonstration of DA modulation of thalamic neural activity, by characterizing the responsivity of the same nuclei to focal dopaminomimetics. S.A.number 3 examines the state-related firing of midbrain DA neurons identified on the basis of their thalamic targets, and the state-related release of DA from functionally homologous striatal regions. These studies are a prerequisite to advancing our understanding of the pathophysiology and treatment of arousal disorders that accompany an array of neuropsychiatric conditions, particularly those which can be broadly defined as hyper- (e.g., schizophrenia) and hypodopaminergic (e.g., PD and restless legs/periodic leg movements of sleep).-

Principal Investigator: SABBAN, ESTHER L
Grant Number: 5R01NS028869-12
Title: Molecular Biology of Norepinephrine Biosynthesis

Abstract: Norepinephrine (NE) is a crucial catecholamine neurotransmitter/hormone mediating a wide range of physiological responses. Alterations in NE neurotransmission are associated with several prevalent disorders, including cardiovascular disorders such as hypertension/hypotension, neuropsychiatric disorders, such as depression and in Parkinson's disease. Regulation of the expression of NE-biosynthetic enzymes, tyrosine hydroxylase (TH) and dopamine beta-hydroxylase (DBH), is a key mechanism of regulation of the NE systems. The specific aims of this proposal are: 1.) Determine the kinetics and the persistence of activation of TH and DBH transcription in rat locus coeruleus with different duration or repetitions of immobilization stress. 2.) Examine the dynamics of pathways involved in transcriptional activation of TH and DBH gene expression in locus coeruleus and adrenal medulla with different durations or repetitions of stress. 3.) Identify the induction of de novo synthesis of transient or long-lasting transcription factors in rat adrenal medulla and locus coeruleus associated with regulation of TH and DBH gene expression by exposure to single and repeated immobilization stress. 4.) Begin to characterize the mechanisms by which the above observed stress responsive factors cross-talk to regulate TH and DBH gene expression. Specifically examine the interaction of AP-I factors and Egr1 on the regulation of TH transcription. The results will provide a crucial understanding of the different transcriptional mechanisms of activation of gene expression of catecholamine producing systems in the CNS and the periphery with acute and repeated exposure to stressful situations. These findings will contribute to the development of new strategies to prevent the harmful maladaptive changes in catecholamine neurotransmission, while enhancing its beneficial adaptive aspects -

Principal Investigator: SALAMONE, JOHN
Grant Number: 1R01NS047261-01
Title: Dopamine D2 and Adenosine A2A roles:Tremulous Movements

Abstract: Symptoms of parkinsonism, such as akinesia, bradykinesia, and tremor, can be caused by degeneration of dopamine (DA) neurons, or by administration of DA antagonist drugs. Parkinsonism is characterized by a cascade of neurochemical events that reflect interactions between several neurotransmitters in the circuitry of the basal ganglia, including DA, acetylcholine, serotonin, GABA and adenosine. Within the last few years, increasing evidence has accumulated indicating that central adenosine neurons play an important role in modulating the functional circuitry of the basal ganglia. Several subtypes of adenosine receptors are involved in motor function, and anatomical studies have demonstrated that the adensonine A2A receptor subtype has a relatively high degree of expression within the striatum. Although several types of striatal cells contain some adensonine A2A receptors, these receptors are present in very high densities on striatopallidal neurons, which also tend to co-express DA D2 receptors and enkephalin. It has been suggested that antagonists of adenosine A2A receptors could have some potential utility as antiparkinsonian drugs. In a recent study from our laboratory, it was demonstrated that IP injections of the adenosine A2A antagonist, KF17837, also suppressed haloperidol-induced tremulous jaw movements, and reversed the locomotor suppression induced by this D2 antagonist. This profile of activity is consistent with the hypothesis that antagonism of adenosine A2A receptors can result in antiparkinsonian effects in animal models. The proposed experiments are designed to investigate the role of the striatopallidal GABAergic pathway as a possible mediator of the putative antiparkinsonian effects of adenosine A2A antagonists. These proposed studies will focus on the tremulous jaw movement model, which is related to parkinsonian tremor. It is hypothesized that adenosine A2A antagonists are acting on striatopallidal GABAergic neurons that also express DA D2 receptors. In view of research showing that haloperidol increases extracellular GABA in globus pallidus, and that haloperidol-induced tremulous jaw movements are reduced by pallidal injections of bicuculline, it is hypothesized that doses of adenosine A2A antagonists that reduce jaw movement activity also will reduce haloperidol-induced increases in GABA release in globus pallidus. In addition, it is hypothesized that adenosine agonists and antagonists will interact to regulate the behavioral and neurochemical effects of haloperidol. These hypotheses will be investigated using studies that involve both systemic and intrastriatal injections of drugs that act upon A2A receptors, and the proposed work will involve a

Principal Investigator: SIDHU, ANITA
Grant Number: 5R01NS034914-07
Title: Dopamine and Oxidative Stress in Parkinson's Disease.

Abstract: Oxidative stress is an important causative factor in the onset and maintenance of several neurodegenerative conditions, such as Alzheimer's disease and Parkinson's Disease (PD). While dopamine (DA)-replacement therapy can control the symptoms of PD, it can also cause severe dyskinesia in patients. Blockage of the D1 DA receptors with Dl-selective antagonists in 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP)-lesioned primates, significantly improves dyskinesia, through unknown mechanisms. Autoxidation of DA is a major source of free radicals; activation of D1 receptors also triggers oxidative stress, and these effects are additive, such that the resulting damage produced in the postsynaptic cell is several fold greater than that elicited by sources of free radicals (hydrogen peroxide (H202)), which does not stimulate the D1 receptor. Several indices of oxidative stress, lipid peroxidation, nitrite production, nitric oxide synthases, neurofilament (NF)-kappaB nuclear translocation, are all elevated two to six fold higher with DA-mediated D1 receptor activation, than H202 alone. We will examine in detail the contribution of D1 receptor stimulation, through the use of agonists and antagonists, in causing oxidative stress in SK-N-MC human neuroblastoma cells, which endogenously express the D1 receptor and is representative of postsynaptic cells. We will examine the mechanism and functional consequences of D1 receptor stimulation on signaling pathways, as well as by selectively blocking parts of the oxidative stress cascade(s). The participation of D1 receptors and oxidative stress in cell death and apoptosis will also be measured. Since blockage of D1 receptors in the MPTP model of PD improves some of the symptoms of PD, we will investigate whether D1 receptors augment MPTP effects in SK-N-MC cells. Conversely, blockage of D1 receptors with antagonists may attenuate MPTP effects on the various indices of oxidative stress. A clear understanding of the effects of dopamine autoxidation and the participation of D1 DA receptors in inducing oxidative stress, is important for understanding patient response to agonist therapy in PD, and may aid in the design of novel therapeutic treatments. -

Principal Investigator: SIDHU, ANITA
Grant Number: 5R01NS041555-03
Title: DOPAMINERGIC SIGNALING IN NEUROLOGICAL DISORDERS

Abstract: Imbalances in dopamine (DA) receptor/G protein coupling dynamics are important in the onset and maintenance of several neuropathological diseases, such as schizophrenia, Parkinson's disease, drug abuse and attention deficit disorder. The D1-like receptors, D1 and D5, share similar structural, physiological and pharmacological homology. The functional attributes of these receptors in DA neurotransmission are largely unknown in diseased and normal states. We have shown that in transfected cells, these receptors can be functionally differentiated in that: D1 receptors couple to both G(s)alpha and G(o)alpha, while D5 couples to G(s)alpha and G(z)alpha. Moreover, D5 but not D1 receptors, inhibit phosphoinositide production. Moreover D1, but not D5, can inhibit adenyl cyclase activity, in the absence of receptor/G(s)alpha coupling. Through functional assays, we will examine the mechanism and functional consequences of D1 coupling to G(o)alpha, and D5 to G(z)alpha, in order to determine whether such coupling causes activation of alternate signaling pathways. Using progressively shorter synthetic peptides directed against specific amino acid motifs of intracellular loops of the D1 and D5 receptor, we will map the domains through which D1 couples to G(s)alpha/G(o)alpha and D5 to G(s)alpha/G(z)alpha. The ability of various peptides to block receptor/Galpha interactions will be examined through co-immunoprecipitation and functional assays. Deletion mutants will be constructed to demonstrate the participation of specific sites in receptor function. We will analyze the interactions between D1 and D5 receptors with their cognate G proteins, using a highly sensitive novel assay, fluorescence resonance energy transfer (FRET). Such FRET studies will enable us to determine in intact cells whether the receptors couple simultaneously to the two Galpha, or if such coupling occurs in a sequential manner. We will also examine interactions between synthetic peptides and G proteins, and determine whether receptor oligomerization is essential for dual coupling of D1 and D5 receptors to Galpha. A clear understanding of the mechanism and functional consequences of coupling of these receptors to different and diverse Galpha is important for defining the roles of these receptors in diseased and normal states, and may aid in the design of novel therapeutic treatments, to selectively activate or suppress specific signaling pathways. -

Principal Investigator: SMEYNE, RICHARD J
Grant Number: 2R01NS039006-04A2
Title: Genetics of MPTP-Induced Parkinsonism

Abstract: Parkinson's disease (PD) is a debilitating neurological disorder that strikes 20 per 100,000 persons greater than 50 years of age. It is estimated that 1 million US citizens have PD, with adults over 60 having a 1 in 20 chance of getting PD. At an average per capita cost of $6000.00 year/patient, the total cost of the disease approximates $6 billion dollars, of which 85% is borne to private and government insurance agencies. Since the population of the world is getting progressively older, the number of people suffering from this disease should substantially increase within the next several decades. The cause of >90% of all PD cases is unknown. Current hypotheses on the etiology of idiopathic PD (IPD) state that there is an interaction of some as yet unknown environmental agent with a genetic predisposition to its effects. The discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has provided a useful model of Parkinsonism that appears to recapitulate the pathology of the disease seen in man. Exposure to this prototypical "environmental toxin" causes a selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). In mice, the effects of MPTP are strain dependent. We have used a QTL analysis to demonstrate that the gene underlying strain differences is located on chromosome 1. Within this chromosomal region, one gene: glutathione-S-transferase pi2 functions within the detoxification pathway for exogenous agents. In this application, we propose to study the structure and function of this gene and its related family members. Four specific aims are proposed: 1) Determine if there are any differences in the sequence and expression of GSTp2 and related family members in MPTP-resistant and sensitive strains of mice. 2) Examine the effects of blockade or transfer of GSTpi on cell death following administration of MPTP in vitro and in vivo; 3) Develop the rotenone model of experimental Parkinsonism in mice and determine if GSTp2 is altered in response to rotenone; 4) Determine if there are structural or expression differences in GSTpi levels in humans with Parkinson's disease. The results of this study should lead to a better understanding of the pathogenesis of experimental and possible human Parkinson's disease. This identification of GSTp2 as a candidate gene could also lead to the identification of diagnostic measures and point to potential therapies for early intervention in this devastating illness. -

Principal Investigator: SMITH, AMANDA D
Grant Number: 1K01NS045698-01A1
Title: Endogenous neuroprotective agents in Parkinson's disease

Abstract: The present application describes the research and career plan laid out for my development into an independent, productive, and well funded investigator in the area of the neurobiology of neurodegenerative disease. The research plan that is proposed investigates the role of circulating insulin like growth factor (IGF-1) and associated proteins in protection of the nigrostriatal dopamine (DA) pathway against oxidative stress induced by 6-hydroxydopamine (6-OHDA) and the nature of this protection. The loss of DA neurons in this pathway underlies the motor dysfunctions observed in patients with Parkinson's disease (PD). Forced use of the impaired forelimb for 7 days in a unilateral 6-OHDA lesion model of Parkinson's disease, ameliorates behavioral asymmetry and restores DA content in the striatum when commenced immediately after or prior to neurotoxic insult. The mechanism by which forced use protects against 6-OHDA toxicity is unknown. Moreover, whether forced use protects the nigrostriatal pathway from degenerating, rescue cells in danger of degenerating in the absence of intervention, or promotes sprouting, is not known. Physical exercise by treadmill or running wheel has been shown to increase the brain uptake of IGF-1 from the circulation and this IGF-1 has been shown to mediate exercise-induced increases in neurogenesis and brain derived neurotrophic factor mRNA in the hippocampus. Thus, it may be surmised that forced use protection is mediated via increases in brain IGF-1 subsequent to increases in circulating IGF-1. Our preliminary data using Fluoro-jade B as a marker of degeneration suggests that forced limb use prevents the nigrostriatal pathway from degenerating. Further, a preliminary screen of altered genes after 6-OHDA and 6-OHDA +/- forced limb use, with microarray analysis suggests that IGF-1 may be involved. In the present proposal, we will: 1) Further examine the impact of forced use/disuse on the anatomical and functional state of DA neurons using behavior, biochemistry and histological analyses; 2) investigate the role of IGF-1 in forced limb use-induced protection, whether this effect can be mimicked by systemic administration of IGF-1 and whether subsequent up-regulation of other trophic factor signaling (i.e. GDNF and BDNF) is involved; and 3) examine whether the protective effects of forced limb use and IGF-1 are mediated via activation of the pro-survival phosphatidylinositol 3-kinase (PI 3K)/Akt and extracellular signal-regulated kinase (ERK) signaling cascades. The career development plan in the present proposal focuses on providing me with the technical skills needed to accomplish the Aims outlined in the present proposal. Further, it will provide the skills and

Principal Investigator: SMITH, YOLAND
Grant Number: 5R01NS037423-07
Title: Metabotropic Glutamate Receptors in the Basal Ganglia

Abstract: The trafficking and anchoring of neurotransmitter receptors to the neuronal plasma membrane are complex phenomena that involve specific interactions between the receptors and various scaffolding proteins. Over the past few years, the targeting of metabotropic glutamate receptors (mGluRs) has gained particular interest. The group I mGluRs, which comprises mGluR1 and mGluR5, are mostly expressed postsynaptically and produce slow depolarization through coupling with phospholipase C and IP3/Ca2+ receptors. Five years ago, Brakeman et al. (1997) identified a dendritic protein that selectively binds to group I mGluRs. The expression of this protein, which was named HOMER, appeared to be regulated by physiological synaptic activity and likely played a role in group I mGluRs signaling. Since then, 12 Homer cDNAs have been cloned. These cDNAs encode for various proteins with a similar structure named Homer 1a/b/c, Homer 2a/b and Homer 3. Although the exact functions of Homer in the brain remain to be established, various data, largely obtained in cultured cells, suggest that Homer is involved in the trafficking and synaptic targeting of group I mGluRs at specific sites along the neuronal plasma membrane. There is strong evidence that changes in dopamine transmission regulate Homer mRNA expression in the rat CNS. The lack of information on Homer proteins localization and their relationships with group I mGluRs hampers the progress of knowledge on Homer/mGluRs functional interactions in the CNS. During the first five years of this award, our laboratory studied in detail the subsynaptic and subcellular localization of group I mGluRs in the monkey basal ganglia. Two major findings stood out from these studies: (1) Group I mGluRs are expressed at both glutamatergic and non-glutamatergic synapses in various basal ganglia nuclei and (2) a large pool of mGluR5 is expressed intracellularly in basal ganglia output nuclei. These observations raised important questions regarding the functions, synaptic targeting, trafficking and regulation of group I mGluRs in the basal ganglia. In order to further characterize these issues and better understand the potential role(s) of Homer in basal ganglia functions, one of the objectives of this proposal is to elucidate the subcellular and subsynaptic relationships between Homer and group I mGluRs in the striatopallidal complex of monkeys. Another goal of this project is to characterize potential changes in the subcellular and subsynaptic localization of group I mGluRs in normal versus Homer knock out mice with or without lesion of midbrain dopaminergic neurons. Together, findings obtained in these studies will serve as a basic framework to understand Homer/mGluRs interactions in normal

Principal Investigator: SMITH, YOLAND
Grant Number: 5R01NS037948-07
Title: GABA RECEPTORS IN THE THALAMUS

Abstract: The basal ganglia and thalamus are interconnected through a series of loops that process and convey basal ganglia outflow to either frontal cortical regions via the ventral motor nuclei or back to the striatum via the caudal intralaminar group, namely the centre median (CM) and parafascicular (Pf) nuclei. Although the existence of a thalamostriatal system has long been established, the role of these projections in the functional circuitry of the basal ganglia remains enigmatic. For the first four years of this grant, we focused our interest on the sources and chemical nature of basal ganglia and brainstem synaptic inputs that control the activity of thalamostriatal neurons. Both the internal globus pallidus (GPi) and the substantia nigra pays reticulata (SNr) provide GABAergic afferents to specific regions of CM/Pf. In addition, the pedunculopontine tegmental nucleus (PPN) is the source of highly heterogeneous chemical inputs to CM/Pf, some of them co-localize GABA and acetylcholine. In addition, neurons in CM/Pf, as in most thalamic nuclei, are endowed with intrinsic GABAergic influences from the reticular nucleus and local interneurons. Electrophysiological data show that GABA plays a crucial role in regulating thalamic activity. However, the exact mechanisms by which GABA mediates its effects on thalamic neurons are complex and still matter of speculation. In order to further characterize this issue, we propose to use state-of- the-art immunocytochemical procedures at the electron microscopic level to elucidate the subsynaptic and subcellular localization of GABA-A and GABA-B receptors in the basal ganglia-receiving territories of the ventral motor thalamic nuclei and CM/PF in monkeys. Abnormal increased GABAergic outflow from the basal ganglia to the thalamus is a cardinal feature of Parkinson's disease pathophysiology. Such increased activity likely results in downregulation of postsynaptic GABA receptors in basal ganglia receiving thalamic nuclei. In order to elucidate this issue, another goal of this project is to compare the pattern of subsynaptic localization of GABA-A and GABA-B receptors in CM/Pf and ventral motor nuclei of normal monkeys and animal models of Parkinson's disease. This series of studies should provide a comprehensive analysis of GABA receptors localization at specific synaptic sites in basal ganglia-receiving thalamic nuclei in primates. Such information is critical for the interpretation of functional studies and a better understanding of the pathophysiological changes generated at pallidothalamic and nigrothalamic synapses in Parkinson's disease.-

Principal Investigator: SMITH, YOLAND
Grant Number: 5R01NS042937-03
Title: GABA-B RECEPTORS AND PARKINSON'S DISEASE

Abstract: Three major receptor subtypes mediate GABAergic inhibitory effects in the mammalian CNS, the GABA-A and GABA-C receptors that generate fast inhibition, and the metabotropic GABA-B receptors (GBR1, GBR2) which mediate slow inhibitory effects via activation of an intracellular second messengers cascade. Data from our laboratory showed that GBR1 receptors are strongly expressed pre- and postsynaptically throughout the monkey basal ganglia. Interestingly, pre-synaptic GBR1 immunoreactivity is mainly associated with glutamatergic terminals suggesting that GABA-B receptors act as heteroreceptors that modulate glutamate release in these structures. To further elucidate the roles of GABA-B receptors in basal ganglia, we propose a series of anatomical, neurochemical and behavioral studies to characterize various aspects of GABA-B receptor localization and functions in the globus pallidus (GP) and subthalamic nucleus (STN) of normal and parkinsonian monkeys. It is well established that overactivity of glutamatergic pathways from the STN to basal ganglia output structures, namely the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr), is a cardinal feature of the pathophysiology of Parkinson's disease (PD). Our preliminary data raise the interesting possibility that activation of presynaptic GABA-B receptors in GP and STN may reduce transmission at overactive subthalamofugal synapses. In this model, activation of GABA-B receptors would attenuate some of the parkinsonian motor symptoms. In support of this notion, our data also indicate that local administration of GABA-B receptor agonists in GPi and STN reduces glutamate release in primates and that systemic application of these compounds may have beneficial therapeutic effects in parkinsonian monkeys. The following four specific aims are proposed: (1) Characterize and compare the pattern of subcellular and subsynaptic localization of GABA-B Ri immunoreactivity in the GP and STh of normal and parkinsonian monkeys, (2) Determine the exact source of glutamatergic axon terminals that express pre-synaptic GABA-B receptors in GP and STN, (3) Test the possibility that local application of GABA-B agonists decreases glutamate levels in the GP and STN of normal monkeys and (4) Test the potential therapeutic effects of GABA-B receptor agonists in parkinsonian monkeys. These experiments will further delineate the subsynaptic localization and roles of GABA-B receptors in modulating glutamate release and open novel research avenues for the potential use of GABA-B agonists in the pharmacotherapy of PD. -

Principal Investigator: SOGHOMONIAN, JEAN-
Grant Number: 5R01NS040783-03
Title: Behavioral Sensitization and Parkinson's Disease

Abstract: The systemic administration of agonists of dopamine receptors remains one of the most effective therapeutic interventions used for the symptomatic treatment of Parkinson's disease. However, the chronic administration of these agonists over several months-years can induce the gradual development of debilitating abnormal involuntary movements such as dyskinesia. Current models of the basal ganglia favor the hypothesis that the chronic administration of dopaminergic agents involves an increased/abnormal GABA signaling in the substantia nigra, pars reticulata (SNr), and in the internal pallidum. We propose to examine this hypothesis in a rodent experimental model of Parkinson's disease. The specific aims are: 1-To test the hypothesis that the chronic administration of agonists of dopamine receptors to 6-OHDA-lesioned rats alters the expression of molecules involved in the regulation of GABA levels in neurons that provide an input to the SNr/internal pallidum; 2-To test the hypothesis that increases in basal extracellular GABA levels in the SNr/internal pallidum are involved in the effects of long-term administration of agonists. 3-To test the hypothesis that plasticity of GABA receptors in the SNr plays a role in the effects of chronic administration of dopaminergic agents. These studies will involve quantitative in situ hybridization histochemistry to measure changes in mRNA levels, microdialysis to measure changes in GABA levels and intranigral administration of pharmacological agents acting on GABA levels or GABA receptors to alter agonist-induced circling in rats unilaterally lesioned with 6-OHDA. -

Principal Investigator: STAROPOLI, JOHN F
Grant Number: 1F31NS048668-01
Title: Parkin and Its Regulation of Neuronal Apoptosis

Abstract: Mutations in parkin underlie an autosomal recessive form of Parkinson's disease, the second most common neurodegenerative disease. To test a working model of parkin as a component of a multi-subunit, SCF-like ubiquitin ligase complex that protects dopamine neurons from apoptosis, other components of the complex, including sel-10 and cullin-1, will be dowregulated by RNA interference in murine primary neuronal cultures. Downregulation of these components will be evaluated for potentiation of dopamine neuron apoptosis and compared to the effects of downregulating parkin itself. To test the hypothesis that a candidate substrate of the parkin-associated complex, cyclin E, is a key mediator of the apoptotic cascade(s) against which wildtype parkin normally protects neurons, pharmacological inhibition of cyclin E-associated activity will be evaluated for rescue of dopamine neurons in the context of parkin, sel-10, or cullin-1 downregulation. Finally, lentivirus-mediated overexpression of parkin in the same primary culture system will be assessed for protection of dopamine neurons from neurotoxins as compared to overexpression of mutant forms of parkin, including clinically defined mutations and forms deleted in the ubiquitin homology and RING domains.-

Principal Investigator: STARR, PHILIP A
Grant Number: 2K08NS002201-04A1
Title: Pallidal Physiology in Human and Primate Dystonia

Abstract: Dystonia is a movement disorder defined as a syndrome of sustained muscle contractions, causing twisting and repetitive movements, and abnormal postures. It is often devastating and its pathophysiology is poorly understood. Recently, attempts have been made to understand movement disorders in terms of alterations in a loop circuit involving the cortex, basal ganglia and thalamus. The globus pallidus internus (GPi) occupies a critical position in this circuit since it is the major output structure of the basal ganglia. Another movement disorder, Parkinson's disease (PD), has been found to be associated with excessive and abnormally patterned GPi activity. This finding has led to improved surgical treatments for PD by pallidal inactivation. In contrast to PD, a better understanding of dystonia has been hampered by a lack of data on the physiology of the basal ganglia in this condition, and by the lack of a well-characterized nonhuman primate model of dystonia. Both problems are addressed in this ongoing study. In the initial three years, we recorded and analyzed 283 pallidal units in 14 patients with dystonia, 74 units in a normal Rhesus macaque, and 75 units from four patients with Parkinson's disease. Human patients undergo electrophysiologic mapping as a routine part of pallidal surgery for movement disorders. We showed that, in comparison with normal macaque, dystonia is associated with reduced neuronal activity in the GPi in most but not all cases, increased bursting activity in GPi, and a slight reduction in activity in the external pallidum. These data lend support to a model of dystonia in which both direct and indirect pathways of the basal ganglia are overactive. However, some cases show little abnormality in discharge rate or pattern, motivating a continued search for a "signature" abnormality in dystonia. In addition, we began development of a model of focal arm dystonia in the Rhesus macaque, in which dystonia is generated by repetitive performance of a skilled motor task. In the proposed continuation, spontaneous and movement-related discharge in GPi will be studied in ten additional dystonia patients, with a new emphasis on neuronal responses to sensory feedback and cross correlation of simultaneously recorded cells. In the macaque model of dystonia, the effect on motor performance of lesioning the globus pallidus will be analyzed. The experiments test the following hypotheses: 1) Idiopathic dystonia in humans is associated with abnormal neuronal synchrony and abnormal responses to somatosensory examination in the GPi. 2) In non-human primates, dystonia induced by a repetitive arm movement task can be ameliorated by lesions of the GPi, establishing the relevance of this model to human

Principal Investigator: STELMACH, GEORGE E
Grant Number: 5R01NS040266-03
Title: Bradykinesia in Parkinson's Disease

Abstract: One of the most debilitating aspects of Parkinson?s disease is the inability to initiate and execute movements as intended. The fundamental hypothesis behind the proposed research is that basal ganglia impairments, as reflected in Parkinson?s disease, causes a disruption in motor programming processes. It is postulated that there is increased noise in the basal ganglia that produce abnormal timing, patterning, and synchronization of discharges into the motor cortical areas. These irregularities in turn reduce motor programming capabilities that are reflected as alterations in the microstructure of a goal-oriented movement. We have documented that Parkinson?s patients produce movements that exhibit shortened primary submovement components, which then requires secondary, corrective movements. Seven experiments are proposed which examine whether the altered substructure of movements observed in PD is related to muscle activation patterns, force-force variability relationships, and/or a reduced capability to incorporate proprioceptive information at different stages of movement planning and execution. The results of these experiments will be evaluated in combination to allow us to determine which of these potential causes has the greatest impact on primary submovement distance. The data obtained will be useful in the basic science realm as well as the clinical setting; the former by isolating the various motor deficits associated with PD, which may allow for inferences to be made regarding structure-function relationships. In terms of clinical relevance these results will assist in identifying where practitioner interventions or outcome measures should be targeted.-

Principal Investigator: STELMACH, GEORGE E
Grant Number: 5R01NS039352-04
Title: MOVEMENT ORGANIZATION DYSFUNCTION IN PARKINSONS

Abstract: While advances in Parkinson's disease have been established in recent years, the pathogenesis of the disease is still not well understood. The primary goal of this project is to quantify how complex multijoint movement is impaired in Parkinsonian patients, thereby providing a better understanding of how motor control principles are compromised. Our working hypothesis concerning PD patients is that much of their difficulty with complex movements arises from their inability to coordinate body segments. We use a trunk-assisted prehension task and analyze trunk, arm, and aperture synchronization when speed, accuracy, sequencing of segments, and visual feedback constraints are imposed. We will analyze body segment synchronization, relative timing, spatial invariance, and synergies. Collectively, the results from these experiments will allow us to better understand how PD affects movement coordination patterns during the performance of complex actions. Comparisons of 'off' vs. 'on' states in Parkinson's disease patients may help determine if coordination impairments share a common levodopa basis. The experiments proposed are systematic, novel and use proven methodology. The proposed research should advance understanding of the fundamental principles that guide the coordination of multijoint movements in normal subjects. It will also increase understanding of the ways in which Parkinson's disease patients are restricted in the use of these principles. The results from four experiments should be useful to both the basic neuroscientist and clinical science communities, reducing the gap between fundamental knowledge of neural mechanisms and therapeutic intervention. -

Principal Investigator: SUN, GRACE Y
Grant Number: 1R13NS047414-01
Title: Conference on Oxidative Mechanisms in Neurodegeneration

Abstract: This application seeks funds for partial support of US investigators to attend a symposium entitled "Oxidative mechanisms in Neurodegenerative disorders" to be held in Guilin, China, August 9-13, 2003. The symposium is a satellite to the International Society of Neurochemistry/Asian Pacific Society of Neurochemistry (ISN/APSN) that will be held in Hong Kong, August 2-7. The Central Nervous System (CNS) is highly susceptible to oxidative stress, which alters many metabolic pathways leading to cellular dysfunction. Since increase in oxidative stress has been implicated in the pathophysiology of a number of age related neurodegenerative disorders, including AIzheimer's disease, Parkinson's disease and stroke, this symposium brings together world-class scientists to share new findings and insights on oxidative mechanisms underlying these neurodegenerative disorders. In addition to sessions focused on oxidative mechanisms associated with Alzheimer's disease, Parkinson disease, stroke, and receptor signaling pathways, a special session will be dedicated to discussing novel methods for invervention and prevention. Thus, this symposium not only provides mechanisms for international scientists and young investigators to focus on an import topic of immense interest to neuroscientists, it also provides opportunities for investigators outside of hina to learn and interact with Chinese investigators in basic and clinical aspects of neurodegenerative diseases. In addition, plans are in progress to provide rapid publication of the symposium proceedings in an internationally recognized neuroscience journal. -

Principal Investigator: SURMEIER, D JAMES
Grant Number: 5P50NS047085-02
Title: Rhythmicity and Synchrony in the Basal Ganglia

Abstract: Parkinson's disease (PD) afflicts roughly 1 in 1000 adults, rising exponentially in incidence after the age of fifty. Human and animal studies have shown that parkinsonism results from the degeneration of the mesencephalic dopaminergic neurons ? In PD patients and in primate PD models, the electrical activity of neurons in globus pallidus (GP) and the subthalamic nucleus (STN) is abnormal. Unlike neurons from normal animals, GP and STN neurons in these animals exhibit synchronous, rhythmic burst discharges. It has been hypothesized that this abnormal activity is responsible for the motor symptoms in PD, providing a rationale for surgical intervention either in the form of pallidal electrolytic lesions or deep brain stimulation of the STN. It is the central hypothesis of this program proposal that the abnormal activity responsible for the symptoms of PD is attributable to adaptations in intrinsic properties of GP and STN neurons and their synaptic interaction following dopamine (DA) depletion. To test this hypothesis, the program brings together four groups with well-established expertise in the electrophysiological analysis of basal ganglia function. The first three projects will use a combination of molecular, pharmacological and electrophysiological approaches to study intrinsic ionic and synaptic mechanisms governing the activity patterns of GP and STN neurons and how these mechanisms are modulated by dopamine. Project 1 (Surmeier) first will generate a molecular and biophysical characterization of voltage-dependent and ligand-gated ion channels governing discharge in identified neurons of the rodent GP and then show how these channels are modulated by dopamine. A combination of !single cell RT-PCR, voltage-clamp and current clamp approaches will be used in acutely-isolated neurons and neurons in tissue slices. Project 2 (Bevan) will provide a similar level of analysis of identified rodent STN neurons using a common set of experimental approaches, in addition to anatomical strategies? Project 3 (Kita) will focus on how STN glutamatergic synaptic input regulates GP neuron activity and how alterations in this input might lead to dyskinesias. These studies will utilize pharmacological, anatomical and electrophysiological approaches in rodents and behaving primates. Project 4 (Wilson) brings these experimental results together to forge biologically grounded compuational model of the GP/STN circuit in normal and dopamine-depeleted states? The successful attainment of these program aims should provide critical information about DA-depletion induced adaptations in basal ganglia neurons most directly linked to the motor symptoms ofPD - placing the neuroscience community in a much better position to devise

Principal Investigator: Tepper, James M
Grant Number: 5R01NS034865-07
Title: Nigrostriatal Dopamine Function

Abstract: The basal ganglia, and especially the dopaminergic components of this system, are well known to play a central role in the role in the etiology and pathophysiology of several neurological and psychiatric disorders including Parkinson's disease and schizophrenia. More recently, however, mesotelencephalic dopaminergic systems have also been viewed as integral to certain types of learning and memory, affective responses and perception, and several types of higher cognitive function. In vivo, dopaminergic neurons fire spontaneously at low rates. This activity exists along a continuum of firing pattern from a regular pacemaker-like pattern on one end, to an irregular or random pattern to a slow bursty pattern on the other end. Dopaminergic neurons in vivo typically respond to behaviorally relevant environmental stimuli with an increase in firing rate in the form of a low frequency burst that usually lasts for a few hundred milliseconds. The timing of the dopaminergic signal is crucial for many of the functions ascribed to the dopaminergic system in signaling stimulus characteristics, reward salience or predictive error. Although it is clear that switches to the different patterns of activity are triggered by afferent activity, the afferents responsible and in particular the mechanisms of the burst or burst initiation are not clear. It is the overall goal of this competing renewal to extend observations made in the last Brant cycle by concentrating on GABAergic mechanisms in the afferent control of substantia nigra dopaminergic neurons studied by in vivo and in vitro neurophysiology, light and electron microscopy and in vivo microdialysis. There are 5 specific aims that will test the following hypotheses: (1) GABA-A receptors on dopaminergic neurons re predominantly or exclusively activated by GABAergic inputs in vivo under typical experimental conditions and activation of GABA-B receptors only occurs when the GABA transporter is saturated by excessive or high frequency Input and/or pharmacological blockade, (2) Most postsynaptic GABA-B receptors on substantia nigra dopaminergic neurons are located perisynaptically, (3) Afferent induced alterations in the pattern of activity of DAergic neurons lead to significant changes in extracellular levels of DA in striatum and substantia nigra, (4) Nigral GABAergic interneurons ore a source of afferent input to DAergic neurons, and (5) The difference in sensitivity to GABA-A receptor agonists between DAergic and GABAergic neurons in substantia nigra is due to a differential GABA-A subunit composition and/or a difference in the density of GABA-A receptors. These data should provide answers to several important questions about the afferent control of nigral dopaminergic neurons

Principal Investigator: TKATCH, TATIANA
Grant Number: 1R21NS048524-01
Title: RNAi Targeting of Kv3 Channels in Basal Ganglia Disease

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by impairment of motor function. It affects about 1 in 1000 adults, rising exponentially after the age of fifty. At present there is no treatment for PD shown to definitively attenuate disease progression. Even temporal correction of symptoms extending the period of physical mobility is considered valuable. We suggest to test a new strategy to relieve motor symptoms of the Parkinson's disease. The abnormal correlated rhythmic activity in the globus pallidus (GP) and subthalamic nucleus (STN) are believed to underlie bradykinesia and tremor of PD patients. A specific set of membrane conductances in GP and STN neurons enable such activity. Recent work by our group has shown that high frequency burst discharge in GP and STN neurons is dependent upon their expression of a combination of voltage-dependent Kv3 K+ channel subunits. These neurons form heteromeric channels containing Kv3.1 and Kv3.4 subunits. These heteromeric channels are very efficient at repolarizing spikes - keeping them very brief - and then deactivating after the spike to allow the next spike to occur quickly. Eliminating the Kv3.4 subunit from these channels diminishes the repolarizing efficiency of the channels, resulting in lower maximal discharge rates. Thus our goal is to test the hypothesis that the suppression of Kv3.4 subunit in GP/STN neurons will dramatically reduce pathological, high frequency burst discharge leading to symptomatic relief in PD models and patients. Kv3.4 is an excellent target for gene therapy approaches since its expression is highly specific for fast spiking neurons and the firing of non-targeted neurons in GP/STN surrounding areas should not be affected. We propose to use lentivirus vector to deliver small interfering RNA (siRNA) designed to trigger the degradation of Kv3.4 mRNA in GP and STN neurons. The proposed specific aims will allow development of the technology that is necessary for testing of our hypothesis in the animal models of PD. -

Principal Investigator: TONEGAWA, SUSUMU
Grant Number: 2P50MH058880-06
Title: Genetic, Physiological, and Behavioral Studies of Memory

Abstract: The long-term objective of the proposed CCNR is to elucidate the molecular, cellular, neuronal ensemble, and neuron circuitry mechanisms of mammalian learning and memory. The CCNR attacks the problem at three levels of complexity: First, to understand the elementary mechanisms of synaptic plasticity and its regulation. Second, to understand how synaptic plasticity subserves single or ensemble neuron activities in the hippocampus and neocortex. Third, to understand how single and ensemble neuron activities represent specific aspects of learning and memory. Further, the Center seeks to integrate findings made at these multiple levels of complexity to reach a comprehensive and coherent understanding of memory. To accomplish these objectives, six investigators from MIT and one from the Salk Institute, who share common intellectual interests and yet come from different fields with complementary expertise ranging from molecular, cellular, and genetic neurobiology to electrophysiology and computational and behavioral neurosciences, will join forces to pursue 30 Specific Aims, many of which are to be carried out in synergistic collaborations. For the elementary mechanisms of plasticity, the Center proposes to apply in vitro electrophysiological recording and fluorescence-mediated imaging techniques to cell-culture and brain slice preparations treated with pharmacological agents or derived from genetically engineered rodents. To understand how synaptic plasticity subserves single neuron or ensemble neuron activities, the Center proposes to use multi-electrode recording techniques in freely behaving rodents. In order to understand how neuronal activities represent encoded, consolidated or retrieved memory information, the Center proposes to study populations of neurons in monkeys and rodents undergoing a specific goal-oriented task. Many projects at the Center use rodents, but monkeys are also used for the study of complex phenomena such as the identification of the neuronal correlates of "executive control" of memory recall. Finally, in order to correlate neural events or processes occurring at multiple levels of complexity with behaviorally manifested memory, the Center proposes to generate mouse strains in which a specific genetic disruption of neural function is spatially and/or temporally selective, and subject them to all the analytical methods described above. Seven Individual Projects are to be supported by three Cores which provide production and maintenance of key mouse mutant strains and administrative services. The Center's basic research is relevant to mental health and illness because mnemonic decline and impairments are a hallmark of aging and neurodegenerative

Principal Investigator: TURNER, ROBERT S
Grant Number: 5R01NS044551-03
Title: DBS AND MOTOR CORTICAL FUNCTION IN AN MPTP MODEL OF PD

Abstract: Deep brain stimulation (DBS) of either the internal segment of the globus pallidus (GPO or the subthalamic nucleus (STN) is an effective treatment for most if not all symptoms of Parkinson's disease (PD). Several aspects of the reduction of symptoms with DBS provide tantalizing hints that different symptoms may be mediated by distinct pathways and/or physiological processes involving the motor and premotor cortices. The goals of this project are to use a non-human primate model of PD to gain a better understanding of the cortical mechanisms by which DBS produces clinical benefit, as well as to determine if different symptoms have different neuroanatomic/physiologic substrates. Animals will perform tasks that measure symptom-relevant behavioral parameters: movement selection/initiation/sequencing (akinesia), movement kinematics (bradykinesia), and rigidity. Neuronal activity at multiple locations in the four principal motor cortices [in different animals, primary motor (M1), ventral premotor (PMv), dorsal premotor (PMd), or mesial premotor (SMA)] will be monitored using a multielectrode array. Single cell activity will be assessed for changes in resting firing rate, task-related activity, and cell-to-cell interactions (synchronized firing) in response to DBS in GPI or STN before and after animals are rendered parkinsonian by intracarotid infusion of MPTP. The predictions are that: DBS-related changes in resting discharge will not be correlated with specific changes in symptoms. Increased activity and synchrony in SMA will be associated with reduced akinesia. Increases of the same in M1 will accompany reduced bradykinesia. Reductions in rigidity will be linked with a drop in M1 responses to passive movement and increased directional specificity in movement related activity. In addition, DBS may reduce abnormally-increased activity in PMv and PMd These hypotheses will be tested in three specific aims: Specific aim I will study the interacting effects of DBS and the type of motor task being performed. Specific aims 2 and 3 will identify cortical activities that change in concert with the time course (SA 2) and parametric relations (SA 3, DBS location, frequency, and strength) of symptom reduction with DBS. The results of these experiments will improve understanding of both the neuronal basis of different symptoms of PD and the mechanisms of action of DBS. Ultimately, these studies will advance a more complete pathophysiologic model of PD by incorporating the full array of parkinsonian symptoms.-

Principal Investigator: VAILLANCOURT, DAVID E
Grant Number: 5F32NS044727-02
Title: fMRI Activity During the Visual Control of Force

Abstract: Functional magnetic resonance imaging (fMRI) at 3 Tesla provides a powerful tool to investigate the sensorimotor processes involved in the neural control of human movement. The long term objective of the investigator is to examine the neurophysiological processes, as measured by blood oxygenation level dependent (BOLD) contrast, involved in the motor control of healthy individuals and extend these paradigms to study the influence of intervention strategies (e.g. rehabilitation, pharmacology) on the physiology of aging and disease. The specific purpose of this proposal is to examine the neural systems underlying the spatial and temporal components of the mechanism that transfers visual signals into motor commands---a visuomotor process. The proposed studies will measure BOLD contrast fMRI and isometric force output from human participants while they perform continuous feedback-based force production. The experiments will examine two hypotheses in two specific aims. Aim 1 tests the hypothesis that the temporal component of the visuomotor process is localized in the parietal cortex and the cerebellum bilaterally. Aim 2 tests the hypothesis that the spatial component of the visuomotor process is also localized in the parietal cortex and the cerebellum bilaterally. It is further hypothesized that the spatial regions within the parietal cortex and cerebellum will be different from the temporal areas shown in Aim1. Collectively, these findings will advance our fundamental understanding of human systems neuroscience and improve feedback models of visuomotor control. These findings will have further implications for better understanding the visuomotor control deficits associated with aging, and diseased persons with Parkinson's disease, ataxia, and cerebellar deficits.-

Principal Investigator: VAN DER WALT, JOELLE
Grant Number: 1L30NS050033-01
Title: Mitochondrial dysfunction in Parkinson's disease

Abstract: Unavailable

Principal Investigator: VEGA, QUINN C
Grant Number: 1R15NS048043-01
Title: Analysis of RET and GFRa-1 down regulation

Abstract: The GDNF/RET/GFRa-1 comlex is involved in many physiological processes including neural migration and neuronal survival. GDNF has also been shown to ameliorate the affects of Parkinson's disease in mice, primates and, most recently, humans. Although RET signaling has been studied in some detail, the mechanism by which this signal pathway is down-regulated after ligand binding is less well understood. Down regulation is critical since it is known that RET, when activated constitutively, leads to unregulated cell growth. It will also be critical to understand, with respect to Parkinson's disease patients, how long term treatment with GDNF affects the signaling pathway and its component parts. The focus of this work will be to monitor RET and the co-receptor prior to and after GDNF treatment with respect to down regulation. Given the importance of these proteins in development, disease and the potential treatment of disease, it will be critical to determine not only how these proteins are activated but what happens after the proteins have been activated. For this project, two potential mechanisms of down-regulation will be measured. With the assistance of undergraduate and master's students, wild type and mutant RET and GFRa-1 proteins will be monitored for changes in transcription, internalization or degradation. Transcriptional regulation will be measured using northern blots and rt-PCR analysis. With respect to internalization, RE'I" and its co-receptor will be monitored using receptor labeling, internalization measurements and co-localization using confocal microscopy of both wild type and mutant proteins. Similar processes will be used to monitor degradation. Finally, once the down-regulation mechanisms have been established, the potential role of disrupted down-regulation in disease progression will be analyzed.-

Principal Investigator: VITEK, JERROLD L
Grant Number: 7R01NS037019-06
Title: Deep Brain Stimulation in the Parkinsonian Monkey

Abstract: Over the last decade, the outlook for patients with advanced parkinsonism and other movement disorders has been revolutionized by the introduction of deep brain stimulation (DBS) in the subthalamic nucleus (STN) and internal segment of the globus pallidus (GPi) as a highly effective treatment modality. According to recent estimates over 2000 patients with PD have undergone implantation of DBS electrodes for the treatment of PD and over 15,000 patients per year may be candidates for this procedure. This number will increase, as the use of DBS as treatment of brain disorders becomes more widespread. Despite their widespread use, very little is known about the physiologic effects of DBS. Given the somewhat similar effect of lesions and stimulation in STN, GPI and thalamus on parkinsonian motor signs, it has been speculated that stimulation may act similar to lesioning, by blocking neuronal activity. Several studies have supported this view reporting suppression of neuronal activity in the site of stimulation. Our preliminary results, as well as the results of other groups have suggested that stimulation may, in fact increase output from the stimulated structure, demonstrating that stimulation in the STN increases neuronal activity in the GPi, while GPi stimulation suppresses neuronal activity in the thalamus. Additional support for this hypothesis is derived from microdialysis studies that found increased levels of glutamate in the entopeduncular nucleus (the rodent equivalent of GPi in primates) during STN stimulation. Conceivably, stimulation of basal ganglia activity may improve parkinsonism simply by regularizing pallidal discharge patterns. Both activation and inactivation could, in fact, be invoked during stimulation, because electrical stimulation may inhibit neuronal activity, while activating fibers in the stimulated area. For further optimization of current DBS protocols, and to minimize risks and side-effects of DBS implantation, it is mandatory that a solid understanding of the mechanism of action of this intervention is developed. This study will determine the mechanism underlying the effects of DBS of STN and GPi by examining in the MPTP monkey model of PD: 1) the effect of stimulation in the STN and GPi on neuronal activity and on neurotransmitter release in different portions of the basal ganglia-thalamocortical circuit, 2) the role of GPe in mediating the effect of stimulation in the STN and GPI, in mediating the development of parkinsonian motor signs and as an alternative site for stimulation for the treatment of PD and 3) determine the effect of stimulation in the STN and GPI on cortical function. The experiments will use a combination of single cell recording, microdialysis, and 18F-fluoro-deoxy-glucose

Principal Investigator: WALKER, PAUL D
Grant Number: 5R01NS039013-04
Title: SEROTONIN CONTROL MECHANISMS OF BASAL GANGLIA FUNCTION

Abstract: (Verbatim from the Applicant's Abstract) Attempts to develop new and effective treatments for movement disorders such as Parkinson's disease have been hampered by an insufficient knowledge of how basal ganglia receptor systems adapt to the consequences of dopamine depletion. This research focuses on determining the role of upregulated serotonin 2A receptors, which we hypothesize provide a mechanism for serotonin to exert greater control over basal ganglia transmission and locomotor function under conditions of dopamine depletion. Our preliminary studies indicate that the target of the serotonin 2A receptor mechanism is the DIRECT striatonigral pathway which utilizes tachykinin neuropeptides colocalized with GABA. New experiments of this application will test the central hypothesis that: upregulated serotonin 2A receptor signaling provides a mechanism for serotonin to enhance striatonigral transmission under conditions of dopamine depletion which influences basal ganglia function and animal behavior. In Specific Aim 1, we will determine the functional consequences of an upregulated serotonin 2A receptor system on serotonin signal transduction within the dopamine depleted striatum by measuring serotonin 2A receptor binding, its linkage to phosphoinositol hydrolysis, its modulation of striatal membrane excitability, and its ability to trans-synaptically regulate striatal tachykinin and GABA expression. In Specific Aim 2, we will determine if tachykinin striatonigral neurons react to the stimulation of upregulated serotonin 2A receptors in the dopamine depleted animal by increasing tachykinin and GABA transmission in the substantia nigra. We will also study the impact of this regulation on locomotor behavior. Finally, in Specific Aim 3, we will determine how an upregulated serotonin 2A receptor system influences the ability of the striatonigral system to regulate basal ganglia dopamine and GABA metabolism, and how these systems influence behavioral recovery of the dopamine depleted animal. Information obtained from these studies will contribute to a better understanding of basal ganglia function and may change how serotonin pathways are considered when designing new pharmacological strategies for diseases which affect dopamine transmission. -

Principal Investigator: WATERHOUSE, RIKKI N
Grant Number: 5R21NS041603-02
Title: Development of PET Radioligands for NMDA Receptors

Abstract: Unavailable

Principal Investigator: WEST, ANTHONY R
Grant Number: 1R01NS047452-01A1
Title: Characterization of Striatal Nitric Oxide Signaling

Abstract: Recent studies have shown that striatal nitric oxide (NO)-producing interneurons play an important role in modulating striatal neural activity and motor behavior. NO is a gaseous neurotransmitter produced by NO synthase (NOS) following glutamate receptor activation. NO diffuses freely through biological membranes and stimulates guanylyl cyclase (GC) and dopamine (DA) release processes critically involved in the generation of motor activity. Studies have shown that striatal NO interneurons receive inputs from the cortex and substantia nigra. However, the influence of these afferents on NOS activity remains to be determined. Additionally, the impact of NO-GC signaling pathways on the synaptic activity of medium spiny neurons (MSNs) is poorly understood. Therefore, the proposed studies plan to examine the afferent systems involved in activating striatal NOS and determine the impact of NO-GC signaling on MSN membrane activity using both in vivo and in vitro preparations. Aim 1 will utilize electrochemical microsensor measures of extracellular NO levels to determine the role of DA receptors in modulating the glutamatergic activation of striatal NOS. Aim 2 will use in vivo intracellular recording techniques in conjunction with microdialysis to determine the influence of NO signaling cascades on the bistable membrane activity of MSNs. Parallel studies will be performed in brain slice preparations to determine the role of GC signaling pathways in mediating the influence of NO on synaptic activity. We hypothesize that activation of corticostriatal afferents will augment striatal NO production in a manner that is differentially modulated by ongoing D1 and D2 DA receptor activation; moreover, activation of NO signaling will increase the excitability of MSNs via a GC-dependent mechanism, in a manner that is potentiated by D1 receptor activation. We believe that these studies will shed light on the mechanisms involved in the integration of dopaminergic and corticostriatal signaling by striatal neurons and suggest novel treatment strategies for Parkinson's disease. -

Principal Investigator: WICHMANN, THOMAS N
Grant Number: 5R01NS040432-04
Title: Influence of subthalamic nucleus on striatal dopamine

Abstract: Degeneration of the dopaminergic nigrostriatal tract results in Parkinson's disease. Over the last years, rodent studies have provided evidence that the activity of the source neurons of the nigrostriatal tract in the substantia nigra pars compacta (SNc) is modulated by afferents from the subthalamic nucleus (STN). Increased STN output, a central feature of most models of parkinsonian pathophysiology, could impact SNc function in early parkinsonism, helping to compensate for the loss of striatal dopamine by increased driving of nigrostriatal neurons. In rodents, STN and SNc are linked via excitatory glutamatergic projections, or via inhibitory pathways involving GABAergic neurons in the substantia nigra pars reticulata (SNr). Activation of the excitatory projections results in increased bursting in SNc, whereas activation of the inhibitory projections lowers the average discharge rates in SNc. Our preliminary data in primates have also demonstrated excitatory and inhibitory effects of STN stimulation on SNc activity, and have indicated that striatal DA levels may be increased with STN stimulation and reduced with STN inactivation. Effects on striatal dopamine may be explained by the direct synaptic STN-SNc interaction, by actions mediated via long loop circuits through thalamus and cortex, as well as by presynaptic mechanisms. The proposed experiments will explore the STN-SNc relationship in primates, with the general hypothesis that STN activation will result in increased burst discharges in SNc and increased dopamine levels in the striatum, while STN inactivation will result in the opposite. A combination of electrophysiologic, microdialysis and anatomic methods will be used to assess effects of transient manipulations of STN activity, induced by intra-STN injections of the GABA receptor agonist muscimol or the GABA receptor antagonist bicuculline, on the neuronal activity in SNc and SNr and on striatal dopainine levels (S.A. V 1/2). Similarly, effects of "deep brain" stimulation and lesions of STN will be studied to assess the impact of these commonly used neurosurgical interventions on SNc and SNr activity, and on striatal DA. In the case of STN lesions, the density of glutamate and GABA receptors in SNc will also be determined (immunoautoradiography) as an inverse measure of the strength of glutamatergic and GABAergic inputs to SNc. These studies will provide insight into the role of the STN-SNc interaction under normal and parkinsonian conditions and will help to understand the mechanisms of action of neurosurgical treatments aimed at SIN in parkinsonian patients. -

Principal Investigator: WICHMANN, THOMAS N
Grant Number: 5R01NS042250-04
Title: Basal ganglia discharge patterns in parkinsonism

Abstract: The basal ganglia are part of larger circuit that involves thalamus and cortex. Cortical inputs reach striatum and subthalamic nucleus (STN), and are transmitted via internal pallidal segment (GPi) and substantia nigra pars reticulata (SNr) to influence the activity of thalamocortical neurons. The function of this circuitry is disturbed in Parkinson's disease because of loss of dopamine in the basal ganglia. Besides changes in discharge rates, basal ganglia neurons also develop significant abnormalities in their discharge patterns in parkinsonism. One of the most salient abnormalities is the appearance of synchronized oscillatory discharge in STN, the external pallidum (GPe), GPi/SNr, and frontal cortex (detected by EEG). Available data suggest that this may result from altered activity along the cortex-STN-GPi/SNrthalamocortical route. With a combination of extracellular basal ganglia recordings and EEG, the proposed primate experiments explore the relationship between oscillatory activity in cortex and basal ganglia and will test the hypothesis that oscillatory discharge in the cortex-basal ganglia circuitry contributes to parkinsonism. The correlation studies under specific aim (S.A.) 1 assess the link between neuronal discharge in the basal ganglia (GPe, STN GPi, SNr) and EEG with simultaneous recordings in both brain regions. The importance of striatal or extrastriatal dopamine loss for the development of oscillatory discharge in parkinsonism will be tested under S.A. 2 by studying changes in oscillatory activity in basal ganglia and cortex induced by microinjections of the dopamine receptor agonist apomorphine at striatal and extrastriatal basal ganglia sites in parkinsonian animals. The experiments under S.A. 3 will test whether blockade of glutamate receptors in STN (blocking corticosubthalamic inputs) reduces oscillatory activity in basal ganglia and cortex. Finally (S.A. 4), the hypothesis will be tested that synchronized oscillatory discharge in the basal ganglia, induced by electrical stimulation of STN with bursts of stimulation pulses at burst rates between 2 and 30 Hz, disrupts motor performance and induces parkinsonian motor abnormalities in normal monkeys. These studies will help to understand the significance of oscillatory discharge in the basal ganglia and cortex in parkinsonism. This may provide guidance in the development of drug treatments directed at normalizing abnormal discharge patterns, and may help to understand the mechanism of action of existing treatments for Parkinson's disease, including dopamine receptor agonists, glutamate receptor antagonists, and deep brain stimulators. -

Principal Investigator: WIEDAU-PAZOS,
Grant Number: 1K08NS002240-01A2
Title: Gsk-3beta & beta-Catenin in pathophysiology of FTDP-17

Abstract: This proposal will enable the applicant to become an independent researcher in the field of inherited neurodegenerative disorders. It builds upon the candidate's background in aging research and implements a comprehensive career development plan that aims to 1) expand the breadth of research skills in the area of cell biology and genetics and enhance current research skills, 2) fill knowledge gaps in the understanding of cellular pathways in frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), and 3) result in publications and academic leadership development. The research will be conducted at the UCLA Department of Neurology with excellent institutional support and opportunities to collaborate. The mechanisms by which mutant tau causes neurodegeneration in FTDP-17 - a group of inherited dementias linked to mutations of the microtubule-associated protein tau - are poorly understood, thereby representing a major knowledge gap in the understanding of cell death pathways in degenerative dementias. The goal of the project is to fill this knowledge gap by focusing on one candidate mechanism, by which tau misexpression may lead to neurodegeneration. We identified this mechanism in previous studies of a Drosophila model of human tau expression. The Aims focus on studies that verify and extend preliminary findings suggesting that GSK-3-beta and beta-catenin, both components of the Wnt signaling pathway, exacerbate mutant tau-induced neurodegeneration related to FTDP-17. Preliminary results suggest that beta-catenin accumulates in CNS regions vulnerable to neurodegeneration and that GSK-3-beta may be sequestered by mutant tau. The applicant will investigate the overall hypothesis that the most common tau mutation, P301L, interferes with the ability of GSK-3-beta to phosphorylate beta-catenin and that the resulting stabilization of beta-catenin triggers enhanced neuronal death. Specifically, correlations of the onset of beta-catenin accumulation and cell death will be addressed. GSK-3-beta activity and association with mutant tau that may lead to beta-catenin accumulation and neurodegeneration will be explored. The proposed biochemical and cell biological studies will initially utilize transgenic mice expressing mutant P301L tau, which model aspects of FTDP-17 clinically and pathologically. Once correlative studies have provided information regarding potential interactions of mutant tau, GSK-3-beta and beta-catenin, functional studies of these interactions are planned. -

Principal Investigator: WIGHTMAN, ROBERT M.
Grant Number: 5R01NS015841-26
Title: ELECTROANALYSIS OF NEUROTRANSMITTERS AND MODULATORS

Abstract: The goals of this research proposal are to develop and use electrochemistry-based probes to monitor neurotransmitters in intact brain tissue. This work is motivated by the belief that monitoring the concentration changes of neurotransmitters in real time provides the most direct way to understand the processes that regulate and control neuronal communication. The neurotransmitter target molecules are dopamine, 5-hydroxytryptamine, and norepinephrine. These molecules are electroactive and are thus a natural target for electrochemical methods. In addition, the three molecules are vital to normal brain function. The information gained by observing these molecules undergoing their job as neurotransmitters will enable their role and their regulation in the brain to be more clearly understood. Furthermore, since real time information is not available for any other neurotransmitters in intact tissue, the information gained will help establish the ways in which neurotransmitters interact with neurons. In addition, we propose to measure local oxygen concentrations and pH. These are both indices of metabolic activity and are closely coupled to neurotransmitter activity. The specific plans of the proposed research are: 1. To improve the response time of carbon-fiber electrodes to neurotransmitter changes. Adsorption of neurotransmitters on carbon-fiber microelectrodes contributes to the sensitivity of these probes in vivo, but it also decreases response time. We propose to characterize the kinetics and mechanism of the adsorption and to minimize the temporal distortion. 2. Design of carbon-fiber surfaces for catecholamine detection. Two surface modification schemes are proposed to improve sensitivity and response time. Carbon surfaces will be exposed to activated carbon to remove impurities and will be covered with quinones. Both methods have been successfully used at large glassy carbon electrodes to improve responses to these neurotransmitters. 3. Probing the regulation of extracellular dopamine. The function of dopamine autoreceptors will be probed to distinguish between autoreceptors regulation of synthesis and release. In addition, we will probe the mechanism of the dopamine transporter in intact tissue. 4. Probing extracellular regulation of norepinephrine. Voltammetric techniques will be used to measure extracellular norepinephrine, an important neurotransmitter in the CNS. The rates of release, uptake, and extrasynaptic diffusion will be studied in several regions of the mouse brain. 5. In vivo electrochemical signals and local blood flow. The carbon fiber electrode can be used to detect extracellular 02 and pH in the brain. 02 levels are related to local blood flow. In this work, we plan to probe the relation

Principal Investigator: Wilson, Charles J
Grant Number: 2R37NS037760-06
Title: Neostriatal Cholinergic Interneurons Firing Patterns

Abstract: Altered function of the neostriatal cholinergic interneurons has been implicated in the pathology of Parkinson's disease, Huntington's disease, and a variety of other disorders. The observation that cholinergic antagonists are clinically effective in treating Parkinson's disease has led many investigators to suggest that within the striatum there is a balance of opposing actions of dopamine and acetylcholine. Despite the explosion of information on the pharmacology of acetylcholine in the neostriatum, physiological information has been difficult to obtain due to the rarity of cholinergic interneurons compared to the other cells in the striatum. Using infrared differential interference contrast microscopy, we have recorded from identified cholinergic neurons in slices, and have shown that they are intrinsic pacemakers that exhibit three distinctly different spontaneous filing patterns, even in the absence of fast synaptic input (but with neuromodulators intact). One of the firing patterns resembles that seen in experimental Parkinsonism. This finding provides a window on several otherwise inexplicable observations, including the rhythmic synchronous activity of these neurons in monkeys rendered Parkinsonian by experimental treatment with MPTP. In the proposed experiments, we will employ whole ceil recording of identified cholinergic interneurons and calcium imaging in single cells to determine (1) The ionic mechanisms of the rhythmic bursting firing mode, which most resembles that seen in Parkinsonism, which we already know is related to modulation of calcium and calcium dependent ion channels (2) The basis for synchronization of cholinergic interneurons when they are firing in the bursting mode, including the synaptic connectivity among cholinergic cells and (3) The influence of D1 and D2 dopaminergic agonists and antagonists on the firing patterns of cholinergic interneurons. The effects of dopamine on firing pattern will be directly related to other studies on dopaminergic modulation of specific ion channels to provide an integrated understanding of the actions of dopamine on cholinergic interneurons and the neostriatal circuitry.-

Principal Investigator: WILSON, SCOTT
Grant Number: 1R01NS047533-01
Title: The role of Usp14 in regulating neuronal function

Abstract: The ubiquitin-proteasome system (UPS) is a central pathway common to all eukaryotic cells for regulating protein turnover. There are numerous regulatory pathways that rely on the timely removal of critical proteins. These pathways include the cell cycle, DNA repair, receptor-mediated endocytosis and the induction of long-term memory. The inability to remove unwanted proteins from cells has been linked to several chronic neurological diseases including Parkinson's disease, Alzheimer's disease, and the Spinocerebellar ataxias. While it is clear that these diseases are associated with polyubiquitinated protein aggregates, it is not clear how these aggregates contribute to neuronal dysfunction. In contrast to the polyubiquitination signal that targets proteins for proteasomal degradation, a monoubiquintin tag can signal receptor internalization and sorting of intracellular vesicles. This modification by monoubiquitin is reversible and, akin to phosphorylation, can regulate protein localization and activity. We have recently demonstrated that Uspl4, a deubiquitinating enzyme (DUB) that specifically removes ubiquitin from proteins, is mutated in the neurological mouse mutant ataxia (ax/j). The axJ mice do not show protein aggregation defects or neuronal loss. Instead, these mice exhibit defects in synaptic transmission, indicating that neurological disease may be rooted in synaptic dysfunction. Our working hypothesis is that loss of Uspl4 disrupts the ubiquitinated state of specific components of the neurotransmitter release machinery, thereby resulting in synaptic defects. This proposal is therefore directed at addressing the role of Uspl4 in regulating synaptic function. The first Aim will determine if Usp 14 associates with the 26S proteasome in neurons and if it has a role in ubiquitin-dependent proteolysis. In the second Aim, we will identify components and pathways that are regulated by Usp14 in order to better understand the regulation of ubiquitin modification in normal physiology and disease. The third Specific Aim will determine which neuronal circuits are disrupted by the loss of Uspl4 and examine how these circuits contribute to the tremor, ataxia and muscle wasting phenotypes of the ax J mice. Completion of these Specific Aims will enable us to uncover new processes that rely on ubiquitin-signaling and to determine how alterations in these pathways can lead to neurological disease.-

Principal Investigator: WU, ALLAN D
Grant Number: 1K23NS045764-01A1
Title: Motor cortex function in unimanual goal-directed aiming

Abstract: This research career development proposal describes a multidisciplinary mentored program allowing the investigator, Dr. Wu, to develop expertise in quantitative motor behavior research methods and transcranial magnetic stimulation (TMS). Dr. Wu will apply this expertise to further the understanding of mechanisms in normal motor control with the long-term goal being to establish how such mechanisms go awry in disease states such as Parkinson's disease (PD), dystonia and sensorimotor stroke. Dr. Wu is a neurologist with current clinical expertise in movement disorders and neurophysiology, who plans to develop a program exploring the research, neuromodulation and eventual treatment potential of TMS in movement disorders. He will be mentored by Dr. Winstein, who brings a quantitative motor behavior approach, as well as access to the support and resources necessary for development of a TMS-equipped neuro-motor physiology laboratory at USC. Drs. Iacoboni and Pascual-Leone will jointly supervise a customized TMS fellowship. Dr. Wu will perform experiments in a practical TMS setting at UCLA under the mentorship of Dr. Iacoboni, and will attend semiannual mini-fellowships at Beth Israel Deaconess Medical Center in Boston with Dr Pascual-Leone. Studies using TMS as a possible therapy for PD, often by targeting TMS over primary motor cortex (M1), have thus far shown inconsistent results, but may be limited by a relative absence of knowledge about how TMS affects normal motor control. Dr. Wu proposes to systematically investigate the effects of TMS over M1 on uni-manual goal-directed aiming movements, a fundamental unit of motor control. Two hypotheses are investigated: 1) The causal importance of M1 in the preparation of discrete aimed movements; and 2) the causal functional asymmetry of M1 for the execution of continuous aimed movements. Results may extend theories of aiming by placing a specific, functionally important, causal role of M1 for aspects of motor control. These studies will form a basis of the outlined career development plan for Dr. Wu from which extrapolation of findings in normal subjects to those with clinical movement disorders (such as PD) may lead to future rational selection of TMS parameters that can predictably and effectively modulate movement disorders symptoms. -

Principal Investigator: YOUNG, ANNE B
Grant Number: 2P50NS038372-06A1
Title: MGH/MIT MORRIS UDALL CENTER OF EXCELLENCE IN PD RESEARCH

Abstract: The MGH/MIT Morris Udall Center of Excellence in PD Research is taking a broad, collaborative and interactive approach to the study of Parkinson's disease. The Projects address critical questions concerning the selective vulnerability of dopamine neurons, the mechanism and consequences of Lewy body formation and alpha-synuclein aggregation, the neural systems consequences of parkinsonism and synuclein pathology, and molecular approaches for modifying this pathology. These issues will be explored using a range of systems, from yeast genetics, to mammalian cell culture, to rodent models to human postmortem material. The Center incorporates state-of-the-art technologies including high throughput yeast genetic screens to identify modifiers of synuclein aggregation and toxicity, viral vector gene transfer to study factors in mammalian cell culture and rodent models, multi-unit tetrode recordings to study striatal plasticity, fluorescence lifetime imaging to study protein-protein interactions, and laser capture microdissection and gene arrays to study transcriptional dysregulation. The Center has a Clinical and Training Core that provides care to patients with Parkinson's disease, gathers data on clinical features of the disease and response to therapy, solicits brain donations for neuropathological study, and trains outstanding clinician scientists to be future leaders in the field. The Center also has a Bioinformatics Core that serves to integrate and analyze data across the projects, and facilitate sharing of the information. The Administrative Core is charged with management of the Center and facilitating the sharing of information, ideas, and reagents among the investigators and with other components of the Udall Centers consortium. The investigators of the MGH/MIT Center are dedicated to a program of collaborative and interactive studies which will lead to better treatments for people with Parkinson's disease.-

Principal Investigator: YOUNG, ANNE B
Grant Number: 3P50NS038372-05S2
Title: MGH/MIT PARKINSONS DISEASE RESEARCH CENTER

Abstract: Unavailable

Principal Investigator: YUREK, DAVID M
Grant Number: 5R01NS042862-03
Title: Gene Therapy, Neural Grafts & Parkinson's Disease

Abstract: Clinical trials have provided encouraging evidence that grafts of fetal dopamine neurons are an effective therapeutic approach toward counteracting the symptoms of Parkinson's disease. Modest therapeutic benefits are observed in grafted patients despite clinical and experimental evidence that survival of grafted cells is low and graft reinnervation is incomplete. The poor survival and limited fiber outgrowth may be a consequence of neural grafts placed ectopically into an environment where the grafted neurons do not receive the proper signals for successful growth and integration into the neural circuitry of the host brain. Gene therapy may be a viable technique to introduce factors [neurotrophic factors] into brain tissue that can potentiate the survival and functional outgrowth of neural grafts, and thus improve the therapeutic value of the graft. In the proposed studies, regulated viral vectors will be injected into the lesioned nigrostriatal pathway of rodents with experimental Parkinson's disease in order to induce transgene expression of several neurotrophic factors that have a history of providing potent neurotrophic support for dopamine neurons. Subsequently, neural grafts will be implanted into lesioned/transduced brain sites and the survival, reinnervation, and function of the grafts will be assessed. Because Parkinson's disease has a higher incidence in the elderly than in the younger population, and recent experimental evidence suggests that the expression of endogenous neurotrophic factors are diminished in the aged striatum following a neurodegenerative lesion, experiments will be performed in young, middle-age, or old rats with experimental Parkinson's disease and the results will be compared within and between each age group. The studies are designed to determine the optimal temporal expression of neurotrophic factors [GDNF, BDNF, FGF-2] that improve graft development and function using regulated viral neurotrophic factors [GDNF, BDNF, FGF-2] that improve graft development and function using regulated viral vectors in young and aged animals with experimental Parkinsonism. These studies will also determine if combinations of viral vectors expressing different neurotrophic factors can be used to improve the therapeutic effects of dopamine grafts.-

Principal Investigator: Ziemba, Kristine S
Grant Number: 1F30NS048716-01
Title: Reconstruction of the nigrostriatal pathway

Abstract: The long-term objective of this research proposal is to develop a means to reconstruct the neural circuit that degenerates in Parkinson's disease (PD) - that is, the nigrostriatal pathway. While current therapy (levadopa treatment) for PD may alleviate symptoms for a while, there is still no way to halt or reverse the neurodegeneration. Since 1% of the population over the age of 65 is affected by PD, and the prevalence increases with increasing age, research into better therapies and an eventual cure for PD is important for our aging population. Cellular replacement is not a new idea in PD research, but this proposal differs from most previous efforts by attempting an anatomically and physiologically correct reestablishment of the nigrostriatal pathway, effecting a more complete behavioral recovery. Molecular cues to guide growth of dopaminergic neurons will be identified in vitro, and adenoviral vectors will be used to express these molecules between the substantia nigra (SN) and the striatum in hemiparkinsonian rats. When dopaminergic neurons are subsequently transplanted into the SN, their axons should grow along the growth-supportive pathway, ending in the striatal target. Success will be evaluated with detailed histological and behavioral analyses.-

Principal Investigator: ZIGMOND, MICHAEL J
Grant Number: 5P50NS019608-20
Title: Neuroprotection and early detection in PD

Abstract: Parkinson's disease (PD) poses a serious threat to the health of a large segment of our society. This is an extensively revised renewal application for a Program Project Grant now in its 18th year. During much of the history of the PPG, we have focused on the compensatory changes that underlie the preclinical phase of PD. That line of investigation will continue, while at the same time we will also add two new foci: first, the development of neuroprotective strategies and, second, the detection of PD it its preclinical phase. Neuroprotection: This will now provide the principal long-term focus of the entire PPG. Our approach derives from recent evidence from our labs indicating that the contralateral motor neglect and loss of DA normally following unilateral damage to the nigrostriatal DA projection can be ameliorated by forced use of the contralateral limb. We hypothesize that forced execution of a motor act that is otherwise compromised by PD is neuroprotective, and that this results from an interaction between the motor act, injury, and concomitant increase in the availability of one or more trophic. We will explore this hypothesis using our 6-hydroxydopamine (6-OHDA) rat model. Our work will involve studies of the role of trophic factors (e.g., GDNF, BDNF, and FGF2), estrogen, and aging, as well as anatomical studies to differentiate between protection, rescue and sprouting (Project 1: M. Zigmond, PI). We also use multineuron recording in awake animals to examine the effect of forced use on the functioning of the basal ganglia more broadly (Project 2, D. Woodward, PI). Compensation: In the past, our studies of compensation have focused our studies on adaptations within the nigrostriatal dopamine (DA) system. Our multineuron recordings will now allow us to explore adjustments within other components of the basal ganglia (Project 2: D. Woodward, PI). Early detection: For neuroprotective strategies to be most effective, it is likely that they must be applied as early in the course of the disease as possible. In this respect, the compensatory changes noted above represent a problem to be overcome through the development of diagnostic tests that can detect PD before the emergence of gross neurological deficits. To do so we will develop a multi-dimensional clinical test battery, using PET imaging as the ultimate criteria for nigrostriatal damage (Project 3, N. Bohnen, PI). We believe that by combining a variety of basic, translational, and clinical approaches we will make significant progress toward the development of a therapeutic approach to PD.-

Principal Investigator: ZIGMOND, MICHAEL J
Grant Number: 3P50NS019608-19A1S1
Title: Neuroprotection and early detection in PD

Abstract: Unavailable